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PHYSIOLOGICAL REVIEWS

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TISSUE CULTURE AND CELL PHYSIOLOGY

ALEXIS CARREL

The Laboratories of The Rockefeller Institute for Medical Research

While the method of tissue culture has been used extensively in morphological studies, its application to physiological problems has been long delayed because the technique was not adapted to the requirements of such investigations. The early procedure (1), which was derived from the experiments of Ross Harrison and is still widely used (2), consisted merely in placing a fragment of tissue in a drop of lymph, plasma, serum, saline solution or other media, hanging in a sealed hollow slide. While surviving under these conditions, the cells are subjected to complex and obscure influences such as those of necrotic cells of their own type, living and dead cells of other types, and a medium which deteriorates spontaneously within a short time. Obviously, the factors responsible for the morphological or dynamic changes observed in the tissues cannot be analyzed accurately. It is as impossible to study a problem of cell physiology with a fragment of tissue in a hanging drop of plasma, serum or other fluids, as it is to investigate the properties of bacteria cultivated in a medium of spontaneously varying composition and contaminated by other bacteria. Before an analysis could be undertaken of the influence of a given type of cells on cells of another type, and of the reciprocal actions of the cells and their medium, more elaborate methods had to be developed. From the early procedure of tissue culture, new techniques were progressively evolved which rendered possible the use of pure strains of cells in a known condition of activity, and of media of almost unvarying composition. Through these important technical improvements, some aspects of the relations of the tissues and the humors of the organism could be investigated. The object of this paper is to review the articles wherein the new techniques and their application to cell physiology have been described.

I. Techniques. *1. Pure strains of cells.* The first step in the adaptation of the method to physiological investigation was the use of pure cultures of cells (3). Long ago, a strain of fibroblasts was isolated from a fragment of embryonic heart (4), (5), (6) and cultivated in embryonic tissue juice which was discovered, at about the same time, to have the property of increasing the growth velocity of fibroblasts and of giving them an unlimited proliferative capacity (7). Pure strains of fibroblasts can be obtained easily from practically every tissue of fowls, dogs, etc., as connective tissue cells multiply more rapidly than the other cells which spontaneously disappear within a few weeks. Then typical fibroblasts alone survive. The pure cultures of fibroblasts (8), which have actively proliferated *in vitro* for nearly twelve years, grow as a dense tissue which doubles its volume in about forty-eight hours. This strain has produced a very large number of cultures during these years, and has been used in many experiments as a physiological reagent for detecting in certain fluids the presence of substances promoting or inhibiting cell multiplication (9).

The isolation of a strain of epithelial cells was attempted many times without success. As the epithelium was always contaminated by a few fibroblasts which grew at greater speed, pure cultures could not be obtained. Finally, Fischer succeeded in growing a strain of epithelium from the few cells of the iris which adhere to the lens of a chick embryo (10). When multiplying on the surface of a plasma clot in a thin layer of embryonic juice, the cells assume the appearance of a continuous membrane. Their rate of growth is slower than that of fibroblasts, and the cultures double in size in about three days. The strain may be kept in active condition almost as easily as a strain of fibroblasts. After more than a year a strain of epithelial cells isolated by Ebeling has lost none of its characteristics or activity. So far, no strain of thyroid, Malpighian, renal or any other epithelial cells has been obtained. Drew has attempted to obtain pure cultures of renal epithelium by protecting an island of epithelium with a drop of mercury and killing the other cells by the rays of a quartz mercury lamp (11). But apparently he did not succeed in keeping this strain permanently. It would be important to isolate pure cultures of glandular epithelium, such as thyroid, and cause them to grow in large amounts for the study of internal secretions.

Pure cultures of large mononuclear leucocytes were obtained from cultures of white blood corpuscles (12). When fragments of a film of leucocytes are cultivated in plasma for a few weeks, the isolation of

lymphocytes and large mononuclears occurs spontaneously. During the first days a large number of polymorphonuclear leucocytes, lymphocytes, and macrophages migrate through the medium. But the polymorphonuclears and lymphocytes progressively disappear. Finally the macrophages alone remain. They multiply slowly in the medium, and wander through it isolated or in columns, but they never aggregate in a tissue (12). The growth of the culture is slow and the cells are less resistant than epithelium and fibroblasts. No strain has been kept for more than three months. But as cultures of lymphocytes and macrophages free from polymorphonuclear leucocytes are obtained easily in a short time, it is unnecessary to keep the same strain indefinitely.

A strain of cartilage cells has been isolated from the *pars cartilago sclerae* of the eye of chick embryos and cultivated for more than three months (13). The hyaline substance disappeared and the small lymphocyte-like cells became transformed into large spindle shaped cells, which formed thin membranes. The rate of growth was slower than that of fibroblasts and epithelium.

So far, no other pure strains have been obtained, although many attempts have been made. Some years ago sarcomatous tumors were cultivated with a view of obtaining a pure strain of malignant cells. Cultures of Rous sarcoma could be kept in active condition for several weeks, but ultimately died (5). The experiments were repeated with mouse and also with human sarcoma (14), and so far have met with the same results.

2. New techniques for the preparation of the cultures. It is well known that embryonic tissue may grow on the fibrin network of a plasma coagulum (1), on the surface of the cover glass in a drop of lymph, serum or saline solution, and on agar (15), (16). But even in a fluid containing all the substances necessary for cell multiplication, no growth occurs if the cells do not find the proper support on which to creep. The finest details of the cells spreading on a cover glass in saline solution may easily be observed, and this technique is excellent for cytological studies. When the rate of growth of tissues must be accurately measured, and when the cultures must be transferred from medium to medium with as little disturbance of their structure as possible, the cells require a scaffold which offers sufficient resistance to handling with instruments (4). Fibrin makes an excellent support for cells which grow incomparably better and more regularly than on agar. The transfer of the tissues from medium to medium is possible without disturbing

the cells, as they are embedded in and protected by the fibrin. The main objection to the use of fibrin is that in the early technique it could be obtained only from plasma, and the serum constituents could not be removed. It was, therefore, impossible to observe the effect of simple saline or other solutions on the growth of cells. This disadvantage has been overcome by a method for obtaining and keeping fibrinogen suspension, or solution (17), which is added to the fluid medium. At first the digestion of the fibrin prevented the use of serum-free media, but this obstacle was met by adding traces of sodium linoleate or egg yolk to the fluid (18). A fibrin coagulum deprived of serum may also be obtained by washing a thin plasma clot in the bottom of a flat flask with Ringer or Tyrode solution (19).

A convenient method was developed for measuring the H ion concentration of the media (20), and the optimum pH for the growth of pure cultures of fibroblasts and epithelium was determined (21).

A medium prepared according to the early technique deteriorates spontaneously within a short time. As the activity of the cells depends in a large measure on the composition of the pericellular fluid (22), no precise relation between a given factor and the changes in the morphology or the growth energy of a tissue can be established if the medium undergoes progressive and unknown modifications. The deterioration of the medium is due to the spontaneous destruction of thermolabile growth-promoting substances contained in tissue juices and serum, and to the accumulation of waste products from the tissues. It is obvious that the tissues used for physiological studies must be kept in a medium of unvarying composition. The ideal method would be to cultivate the cells in a continuous stream of oxygenated medium. Although an attempt was made to circulate serum at the surface of, or through, a plasma clot (5), (23), no practical method has been evolved from those early experiments. The first technique by which cells could be kept indefinitely in a condition of constant activity consisted in removing the tissue fragment frequently from its medium, washing it in Ringer solution, and transferring it to a fresh medium (4), (5). As the nutritive substances contained in the medium do not undergo marked disintegration in forty-eight hours, and as the accumulation of waste products has but a very slight action on the growth of the tissues during that period of time, the cultures, when transferred from medium to medium, live under practically unchanged conditions (9). This method has several disadvantages, namely: it is laborious and requires training to extirpate the tissues from the medium and

transfer them to a new medium; only a small amount of tissue can be handled; and some tissues do not stand the passages well. While strains of fibroblasts and epithelial cells can be maintained indefinitely in an almost constant condition of activity, macrophages do not resist the frequent passages as easily as other cells. Furthermore, cell secretions which require the cultivation of large amounts of tissue under constant conditions cannot be studied.

A second technique has been developed by which the culture medium may be renewed frequently without mechanical disturbance of the tissues or danger of bacterial contamination (19). Special containers have been made consisting of flat, round flasks with oblique necks. They belong to different types, some being used for measuring the rate of growth of tissues, and the others when cytological examinations are required. The size of the flasks varies from 5 to 8 cm. in diameter, which makes possible the use of large amounts of tissues and medium. By means of special instruments the tissues may be handled conveniently inside the flasks. The principle of the method is to use a medium composed of two parts, one solid and the other fluid, the latter being renewed as often as necessary. The solid medium is composed of coagulated plasma in which the tissues are embedded and which covers the bottom of the flask in a thin layer. The fluid medium, which bathes the solid medium continuously or not as desired, is composed of Tyrode solution in which are dissolved the substances whose action on the tissues is being investigated. The fluid medium diffuses its nutrient principles in the coagulum and also receives the catabolic products of the tissues. As it can be changed every day, the tissues grow on the bottom of the flask, without being disturbed, in a medium which varies but little in composition. With this technique, epithelial cells, fibroblasts and leucocytes may be observed in a condition of uninterrupted growth for about a month.

3. Techniques for measuring the rate of growth of a tissue, detecting growth active substances in a fluid, and ascertaining the inherent growth energy of cells. As the action of a given substance on a tissue must be measured by its effect on the growth of a pure strain of cells, it was ascertained whether two parts of a tissue fragment may be caused to grow at identical rates in identical media. A minute study was made of the technical conditions which are required for the uniform growth of pure cultures of fibroblasts (24). When the technique is carefully carried out, the differences in the rate of growth of fibroblasts in identical media are slight and do not exceed 7 per cent. But

the technique is delicate and, in untrained hands, the experimental errors are of such magnitude as to render the results worthless.

The rate of growth of pure cultures of fibroblasts and epithelium is measured by a technique developed recently (24). As the mass of the tissues is too small to be accurately weighed, an indirect method must be used. When the cultures are correctly prepared, the thickness of the growing tissue is uniform, and the increase in area of the culture may be considered as expressing the increase in mass. The measurement of the rate of growth is made in the following manner. The slides or flasks are placed in a projectoscope and the outlines of the tissues are drawn immediately after the preparation of the cultures, and after forty-eight hours' incubation. The areas of the original fragment and of the new growth are measured with a planimeter and the ratio of the area of the new growth to that of the original fragment is calculated. This relative increase of pure cultures of fibroblasts and epithelium in forty-eight hours expresses the rate of growth. The rate of growth, the increase in mass of the tissues in a number of passages, and the duration of life of the cultures indicate whether the medium is nutrient, non-nutrient, or growth-stimulating without being nutrient (19). When the tissues are cultivated in large flasks, the increase is measured in the same way and, as the growth is uninterrupted, it may be expressed graphically in a simple manner (19). The curve expressing the rate of growth of fibroblasts and epithelial cells in a nutrient medium is a parabola. If the medium is non-nutrient, the curve is S-shaped. Modifications in the form of this curve indicate whether the medium contains substances which activate or depress the rate of cell multiplication. The technique cannot be used for measuring the activity of lymphocytes because they never grow as a tissue, but always remain at certain distances from one another and invade the culture medium on several planes. With this method of measurement, the action of a given substance under a known concentration on the rate of growth of a pure culture may be ascertained with some accuracy. It is, then, possible to use strains of epithelial and connective tissue cells as physiological reagents in order to detect in the humors the presence of substances promoting or depressing cell multiplication, and to express the action of these substances in terms of unit area of growth.

The inherent growth energy of a given tissue can also be ascertained (25). It is obvious that the growth energy of a tissue *in vivo* may undergo wide variations under the influence of several factors and

chiefly of age. The curves of Minot demonstrated this long ago (26). The growth energy of the fibroblasts of a given organ is greater in an embryo than in a young animal, and fibroblasts in a nutrient medium are more active after twenty-four hours than after seventy-two hours. It is also known that a tissue may increase in growth energy by cultivation in a medium containing a large amount of embryonic substances (22). Therefore, the inherent growth energy of a tissue at a given instant is a function of several independent variables: its growth energy at the preceding instant, and the concentration in the pericellular fluid of the substances which increase or decrease cell activity. It could be ascertained by the activity displayed during an instant by the cells in a medium containing neither activating nor retarding substances, if such a measurement were possible. But the residual energy, which was found to vary at the same time as the inherent energy, may be used as a measurement of the latter. The residual energy is expressed by the duration of life and the proliferative activity of the cells in a medium containing no nitrogen (25).

II. Application of the techniques to physiological problems. *1. Origin of the nitrogen used by fibroblasts, epithelial cells and macrophages in the synthesis of protoplasm.* In the early studies (1), (5), (27) the tissues were supposed to live on the constituents of the lymph or plasma used as a culture medium. But soon it was found that proliferation of embryonic tissues takes place as readily in saline solution (15) as in serum, and that the tissue fragments cultivated in plasma do not increase in mass (28). Although the minute fragment of embryonic heart from which originated the first pure strain of fibroblasts proliferated for more than three months in plasma, it progressively decreased in volume until embryonic juice was added to the medium (5). These and other experiments led to the conclusion that cells cultivated in plasma do not feed on the serum constituents but on substances contained in the tissue itself (15), (16), (28), (29). Recently, the function of plasma and serum in the nutrition of fibroblasts was ascertained in extensive series of experiments (18), (30) made on pure cultures of fibroblasts with both techniques, repeated passages of the tissue in the medium, and changing the medium while the tissue grows undisturbed in a flask. These experiments rendered evident the fact that fibroblasts live no longer in Tyrode containing different dilutions of serum than in pure Tyrode solution. When the amount of serum is large, the cells die sooner than in saline solution. If a small quantity of embryonic tissue juice is added to the medium, the growth of fibro-

blasts and duration of their life is in no way influenced by the presence or absence of serum. Even if embryonic juice contains some growth-stimulating hormones, these hormones fail to enable the fibroblasts to use the nitrogenous constituents of serum. But fresh embryonic heart can live in plasma much longer than a pure culture of fibroblasts because the disintegrating tissues set free substances which are used by fibroblasts for the synthesis of protoplasm (18). The proliferation of cells cultivated in plasma depends on some nitrogenous material stored within the cells and the tissues themselves. Serum proteins are not used by fibroblasts as a source of nitrogen (18). Therefore, plasma and serum cannot be considered as a culture medium for those cells, in the proper sense of the word.

Several other fluids were supposed to be nutrient for tissues *in vitro*. Chicken bouillon is a part of the medium called Locke-Lewis solution, but it cannot be used by fibroblasts any more than serum (31). In some experiments made with the early technique, the action on fragments of embryonic heart of a number of amino acids was found to be toxic (29). But it was disclosed recently that amino acids under the same concentration as in the blood have no poisonous effect on fibroblasts and epithelial cells in pure cultures, and that some of them increase the rate of cell migration and multiplication. But they are not used as a source of nitrogen by the tissues, which die as rapidly as in pure Tyrode solution. Egg white allows a longer survival of the cells, and sometimes the fragments of tissue increase slightly in size. Crystallized egg albumin in a concentration similar to that of albumin in the serum does not allow an increase in mass of the connective tissue (31). These experiments definitely proved that fibroblasts and epithelium do not synthetize protoplasm *in vitro* from nitrogenous constituents of plasma, serum, bouillon or amino acids, and that in these media they are in a condition of survival and not of cultivation (31).

Fibroblasts and epithelial cells may be considered as in a true state of cultivation only when embryonic tissue juices are present in the medium (7), (22), (30). Whenever embryonic juices are added to the medium of fibroblasts or epithelium, the mass of the tissue begins to increase. As long as the cells are supplied with embryonic juice, they synthetize new protoplasm and build up unlimited amounts of tissue. According to early experiments (7), and also some recent ones, it seemed that extracts of sarcoma, adult muscle and glands, leucocytes (32), (34), and even of heterologous tissues, were endowed with a growth-promoting action similar to that of embryonic juices (33), (35).

A recent article reported the results of a study of the action of the juices from homologous and heterologous tissues on pure cultures of fibroblasts, not only for a few days, but for long periods of time (36). The effect of these juices, which may be practically similar for a few days, differs widely when their action is prolonged. Extracts of adult chicken organs, like embryonic tissue juice, at first determine an increase in the mass of the pure cultures of chicken fibroblasts, but, after a few passages, the fibroblasts cease to multiply and ultimately die. It is probable that adult tissues, as well as serum, contain substances which are toxic for the homologous cells, and progressively overcome the effect of the growth-promoting compounds. The activating effect attributed recently to adult gland and sarcomatous tissue by Drew (35) is a confirmation of the early experiments (7). There is no marked difference between homologous and certain heterologous adult tissue extracts in their effect on fibroblasts. By contrast, heterologous embryonic juices may act on chicken fibroblasts in the same manner as chick embryo juice. It appears that fibroblasts can synthetize protoplasm from substances contained in certain foreign embryonic juices (36).

Several years ago aqueous extracts of sarcoma were found to enhance cell multiplication (5), apparently in the same way as embryonic tissue juice does. This fact has recently been confirmed (35), (36). But the action of extracts of sarcoma is more complex than was thought at first. When chicken tumor extracts are added to the culture medium for several passages, the rate of cell multiplication, which is at first increased, becomes slower and the fibroblasts may ultimately die. Therefore, the action of the juices from tumors can be compared only in a superficial way to that of embryonic tissue juices.

While fibroblasts and epithelial cells remain in a true condition of cultivation only in homologous and certain heterologous embryonic tissue juices, macrophages behave differently. When they are cultivated in embryonic tissue juices, they display great activity at first, but die after a short time. On the contrary, if the medium is composed of diluted serum, they remain alive and spread over a large area for more than a month (37). It is obvious that they build up protoplasm from some constituents of serum, while neither fibroblasts nor epithelial cells possess this property. Thus, it appears that the nitrogenous compounds required by tissues for the synthesis of protoplasm may vary according to the nature of the cells, and that the nutritive requirements of epithelium and fibroblasts cultivated *in*

vitro differ from those of macrophages. It is not proved that such striking differences exist within the organism. Cell nutrition is not identical *in vivo* and *in vitro*. *In vitro*, plasma disintegrates into fibrin and serum. Serum is an artefact. Tissues within the organism may continuously receive substances which circulate in very high dilution in the blood, while *in vitro* these substances are soon exhausted and cannot manifest their action. However, there is some reason to believe that in the adult animal, connective tissue does not find in plasma the substances necessary for its multiplication. If they were capable of proliferating in blood plasma, endothelial cells would soon fill the lumen of the vessels. Connective tissue is at rest in practically every part of the adult organism, probably because it does not find in interstitial lymph and plasma the necessary growth-promoting substances. In the adult organism, fibroblasts do not proliferate spontaneously, but they multiply around foreign bodies or regenerating structures, when leucocytes secrete, or necrotic cells set free, substances similar to those which are found in tissue juices. Should nutritive conditions be analogous *in vivo* and *in vitro*, epithelium and fibroblasts, which cannot multiply in serum, would use for their proliferation certain nitrogenous compounds which are synthetized from serum by leucocytes and possibly by other cells. These substances would be the substratum of the formative stimulus which, according to the pathologists, imparts to the tissues their regenerative growth energy.

2. Properties of the growth-promoting substances contained in embryonic tissue juice. The nature of the nitrogenous compounds present in embryonic tissue juice and used by epithelial and connective tissue cells in the building up of protoplasm is still entirely unknown. But some of their properties have been studied during the last twelve years. Embryonic tissue juice loses its growth-promoting effect after it has been shaken for several hours, or heated at a temperature of 65°C. for a few minutes (7). The substances which promote cell multiplication are as sensitive to heat as alexin and sensitizer. They are partly destroyed at 56°C. in thirty minutes (7), and when left in an incubator at 38°C. for forty-eight hours, their action is greatly weakened. They are unstable and easily destroyed by agents other than heat. They are sensitive to the action of radium and x-rays. Spontaneous deterioration occurs also under the influence of still unknown factors. The decrease in the pH, which occurs spontaneously even at low temperature, prevents their stimulating action (36). But even when the pH does not vary, embryonic juices which have been

kept in the refrigerator for one or two weeks lose some of the growth-promoting power (36). Filtration through a Berkefeld filter partly deprives the juices of their growth-activating substances, and these substances disappear completely after filtration through a Chamberland filter (7). These facts have recently been confirmed (11). Many attempts have been made to isolate from the embryonic tissue juices the substances used as a nitrogenous food by fibroblasts. Fractionate precipitation by alcohol, acetone and other substances did not lead to the separation of a more active moiety. The growth-activating substances always remain with the protein precipitate. The term, "growth-activating substances," is merely used for the sake of convenience and applies to a complex group of substances. This group consists probably of some growth-stimulating hormones of the class called by Gley harmozones, and of other substances which supply fibroblasts and epithelium with the nitrogen required for multiplication. These nitrogenous compounds, endowed with the property of being used as food material by certain tissues, may be called trephones, from τρέφω, I feed.

3. Function of leucocytic secretions in the nutrition of other cells. The presence in embryonic tissue juices and leucocytic extracts (32) of substances which contain nutrient principles used by fibroblasts and epithelial cells suggested the possibility that these substances are also present in the secretions of embryonic and other cells. So far, it has been technically impossible to ascertain whether thyroid or ovarian cells living in pure cultures secrete the nitrogenous compounds required by fibroblasts and epithelial cells. But the properties of leucocytic secretions could be investigated (38). Fragments of a film of white corpuscles obtained from chicken blood were cultivated in flasks. After a few days the serum obtained from the cultures containing the leucocytes in active condition was found to promote cell multiplication to a greater extent than that of the controls. Simultaneously, the natural hemolytic action of the serum increased in the cultures containing active leucocytes. When a small amount of casein was added to the medium, the leucocytic secretions acquired a still greater growth-promoting power. It was evident that living leucocytes, spontaneously or under the influence of a foreign protein, secrete simultaneously substances which possess the same growth-activating power as embryonic juices and also natural hemolysins (38).

Further experiments have been undertaken in order to ascertain whether leucocytes secrete growth-promoting substances, not only

in vitro but also *in vivo* (32). Aqueous extracts of inflamed connective tissue were found to have the power to increase the rate of fibroblast proliferation. A peritoneal exudate containing many leucocytes also had a marked activating action on the growth of connective tissue. Then, leucocytes can set free *in vivo* the growth-promoting substances which they manufacture (32). They may be considered as storing the nitrogenous food required by fibroblasts and leucocytes or, in other words, as being the nurses or trephocytes of fixed cells. Since lymphocytes are capable of multiplying in serum, it is probable that they synthetize growth-activating substances from certain constituents of serum. Colonies of lymphocytes and fibroblasts were embedded in plasma in a large flask, and cultivated in serum. After a few days the fibroblasts were in a condition of apparent death. But as they were reached by the macrophages, which were multiplying actively, they soon became rejuvenated. Apparently, the macrophages obtained from the serum the material from which they manufactured the nitrogenous compounds necessary to fibroblasts (37). Small and large lymphocytes may bring to fixed cells the substances required for their proliferation and supply adult tissue with the substratum for cicatrization and regeneration.

4. Cell dedifferentiation and differentiation. Long ago Champy observed that epithelial cells which migrate from fragments of kidney surviving in a drop of plasma lose most of their characteristics (39), and return to an indifferent stage where epithelial and connective tissue cells cannot be distinguished from one another. He found also that the specific secretion of the guinea pig prostatic cells, when cultivated in serum, soon disappeared (40). When connective tissue was present, dedifferentiation of epithelial cells did not occur (41). He concluded that epithelium cultivated *in vitro* is bound to dedifferentiate when no connective tissue is present. More recently, the same theory was defended by Drew (11), (35). It is hardly possible to infer from those experiments, which were made according to the early technique and with impure cultures, that cells cultivated *in vitro* necessarily dedifferentiate. In Champy's experiments the tissues were not in a condition of cultivation, but merely of survival, and the duration of survival was very short. Those experiments should be repeated with pure cultures of renal cells and fibroblasts.

Dedifferentiation of epithelial cells in tissue cultures is far from being a necessary consequence of life *in vitro*. On the contrary, it appears that a pure strain of epithelial cells can maintain its essential character-

istics after a long period of cultivation. A strain of epithelium isolated from the iris has grown as a pavement membrane and secreted a large amount of pigment for more than a year. These cells have evidently remained epithelial in character, although no connective tissue is present. This fact shows that, when pure strains are cultivated in media which do not deteriorate rapidly, results very different from those brought about by the early technique may be obtained.

When strains of epithelial cells and fibroblasts are cultivated side by side in the same medium, they keep their individual characteristics. No transition takes place. The connective tissue cells promptly surround the epithelial cells which aggregate in glandular-like structures (42). This fact has been recently confirmed in other experiments (11), (35). The cultivation of complex tissues may facilitate the study of the interactions of cells of different types under various conditions. Small bodies made of cylindrical epithelium, containing epithelial, connective and muscular tissues, endothelium and amoeboid cells, were cultivated in suitable media. Pure cultures of epithelial cells could be grafted into this artificial organism. The medium supplied the tissues with sufficient nourishment through the intestinal epithelium (43).

It is known that lymphocytes cultivated in plasma increase in size and become transformed into macrophages (34). Lately, macrophages were found to differentiate into fibroblasts (12). Fibroblasts generally appear in a culture of white blood corpuscles when a contraction of the coagulum brings together masses of macrophages. From the apparently necrotic material, elongated cells sometimes grow, and typical fibroblasts and intermediary forms between fibroblasts and macrophages are observed (12). But the determining factor of the transformation of macrophages into connective tissue cells could not be ascertained precisely. In the flasks where macrophages spread evenly, no transformation of mononuclear leucocytes into fibroblasts took place (37).

5. Action of a foreign protein on tissues. Casein inoculated into a culture of leucocytes brings about an immediate increase in the substances which promote the growth of fibroblasts and natural hemolysins. The cells respond immediately and non-specifically to the foreign substances (38). The specific reaction of leucocytes to an antigen was found to appear after four days (44). Fibroblasts respond also to the presence of a foreign protein in the medium and become immunized in about four days (45). There is a relation between the amount of antigen, the time of the appearance of the immunization and its duration (46).

6. Depressing action of serum on cell proliferation. Serum not only fails to supply fibroblasts and epithelial cells with the nitrogen necessary for multiplication, but inhibits their proliferative activity and shortens their life *in vitro* (47). Although lymphocytes find in serum the substratum for multiplication, their activity is depressed by a large amount of this fluid in the medium (12). The apparent acceleration of growth which occurs when plasma is diluted with Ringer or Tyrode solution is caused merely by a decrease of the inhibiting action of serum under the influence of dilution. Although this effect was studied long ago (48), (49), (50), its mechanism had remained unknown until the discovery of the growth-inhibiting action of serum (47).

The restraining action of serum on cell multiplication becomes more pronounced after the serum has been shaken for one hour or more (51), or subjected to the action of heat at 65°C. for thirty minutes (52). Serum kept for two days at 38°C. becomes more inhibiting. Radium and x-rays increase the depressing action of serum on tissue growth. As a rule, the agents which inactivate serum render it more inhibiting for homologous fibroblasts. It seems that serum contains certain growth-promoting substances which, like alexin and sensitizer, are destroyed by shaking and heat, and oppose the inhibiting effect on fibroblasts of other substances which resist heat and shaking (52). A direct proof of the presence in serum of antagonistic principles was given in the following experiments (53). A precipitate, obtained by bubbling CO_2 through serum, was found to have an activating effect on fibroblasts, while the remaining part of the serum had become more growth-inhibiting than the complete serum. Thus, it appeared that serum contains two groups of substances, the growth-activating ones always being weaker in their action than the growth-inhibiting (53). The growth-promoting substances of serum are as unstable as those contained in embryonic juices and leucocytic secretions, and possibly are set free in the blood by leucocytes and certain glands.

Serum never activates the multiplication of fibroblasts, even when it is obtained from a very young animal (47). But the plasma of a six-week-old chicken has no depressing action on homologous fibroblasts. The growth-inhibiting power of serum is already apparent in the three-month-old chicken, increases progressively with age, and becomes very marked in old animals. While the life of fibroblasts in the plasma of a nine-year-old hen lasts for four or six days, its duration is about fifteen days in that of a three-year-old fowl, thirty days in that of a three-month-old chicken, and forty-six days in that of a six-

week-old chick. The rate of cell multiplication and the duration of life in plasma decreases in function of the age of the animal from which the plasma is obtained, at first very rapidly, and in late years very slowly (47). The curve expressing this phenomenon resembles the growth curves of Minot and the curve of the index of cicatrization of du Noüy's formula in function of the age of the patient (54). As no growth-activating action can be detected in the serum of very young animals, it appears that, during the whole life, the growth-inhibiting substances contained in plasma have a stronger effect on fibroblasts than the growth-activating ones, and that the effect of the latter is entirely shielded by that of the former.

It is probable that the properties of both antagonistic groups of substances become modified in the course of life, and that the enhanced restraining action of serum in old age is caused by a decrease in the activating substances, or by an increase in the inhibiting ones, or by both (55). Should the growth-activating principles of serum be more abundant in youth than in old age, their destruction by heat would increase the inhibiting action of the serum of a young animal relatively more than that of an old animal. This supposition was subjected to an experimental test, and the serum of young chickens was found to inhibit cell proliferation relatively more than that of old chickens, after both had been heated at 65°C. (55). There are, then, more activating substances in the serum of youth than in that of old age. But after the destruction of these activating substances, the inhibiting action of the serum of the old animal is still greater than that of the young one. Another verification of the hypothesis was sought by examining the CO_2 precipitate obtained from the sera of young and old animals. The precipitate from the serum of a young animal definitely increased the proliferative activity of homologous fibroblasts while that from the serum of an old animal had practically no activating power. After the precipitate had been removed, the serum of the old animal was still more inhibiting than that of the young one (55). The increased inhibiting action of serum from old animals on homologous fibroblasts appears to be due to the loss of growth-promoting principles and, at the same time, to the enhanced power of the growth-inhibiting principles. The growth-promoting substances probably come from gland secretions, and it is obvious that these secretions decrease in old age. The enhanced growth-inhibiting effect may be due to a change in the properties or the quantity of the serum proteins. It is known that the concentration of proteins in rat serum increases

with the progress of age (56). The same phenomenon occurs in fowls. But when the serum of an old fowl is diluted so that its protein concentration is practically equal to that of a young fowl, its growth-inhibiting power, which has decreased markedly, is still higher than that of the young animal. While increase in protein concentration is one of the mechanisms by which age acts on the inhibiting power of serum, other factors certainly play a part in the phenomenon.

The property of the blood serum of becoming more growth-inhibiting with advancing years may be used as the basis of a technique for measuring age (57). The ratio of the rate of cell proliferation in serum to that in Tyrode solution is called growth index. It seems that it varies as an exponential function of the time. The value of the growth index decreases rapidly during the first months of life and slowly in old age. As chicken fibroblasts respond to the inhibiting power, not only of homologous, but also of certain heterologous sera, they may be used as a reagent for the detection of the modifications brought about by age in the blood of dogs. When the growth index is adjusted to be 1.00 in dogs about two weeks old, it becomes rapidly lower and variations from 1.00 to 0.70 and even 0.50 can be observed within two years. On the contrary, in old animals the variations of the growth index are small. In animals eight or nine years old, its value varies generally between 0.2 and 0.1. In still older dogs, the technique is not sensitive enough to bring about differences larger than these which may be due to experimental errors.

CONCLUSIONS

During the past years the method of tissue culture has been progressively adapted to the requirements of physiological research. For the early procedure, which allowed merely a short survival of the tissue in a deteriorating medium, were progressively substituted more elaborate techniques permitting the cultivation, in the true sense of the word, of cells of a given type in media of constant composition, and under such conditions that the rate of growth could be measured. The more important steps in the development of the method were: the isolation of pure strains of fibroblasts, epithelial cells, and monocytes; the discovery of the property of embryonic tissue juices for promoting unlimited proliferation of certain cells, the techniques by which tissues could be maintained alive indefinitely, and their rate of growth measured.

Then, it became possible to detect in the humors of the organism and other fluids the presence of certain substances which act directly on cell nutrition, and to ascertain the inherent growth energy acquired by a tissue, either *in vivo* or *in vitro*. The techniques are still in the early stages of their development, and must be used with great care to yield accurate results. Although they have been applied so far to few problems, some new facts have already been brought to light:

Fibroblasts are endowed with an unlimited capacity for growth. They proliferate as long as they are supplied with the proper nutritive medium. Their growth-energy does not depend on that originally received from the ovum, but on the presence in the pericellular fluid of certain substances which are found in embryonic juices and in leucocytic secretions. These substances differ from the hormones because they take part in the synthesis of protoplasm, and do not act merely as a catalyst of growth. They are called trephones. Epithelial and connective tissue cells do not build up protoplasm from serum. Lymphocytes and large mononuclear leucocytes can live in serum and manufacture from its constituents growth-promoting substances. They are capable of being the nurses or trephocytes of fibroblasts and epithelial cells. Serum has a depressing effect on the proliferation of fibroblasts and epithelial cells and this effect increases markedly with the age of the animal. The growth promoting substances, or trephones, have some relation with alexin and sensitizer. Both groups of substances may undergo simultaneous variations due to the same cause. The factors which decrease the growth-inhibiting effect of serum on homologous cells generally increase its destructive power on foreign cells at the same time.

These results indicate that the techniques which have slowly evolved from the early procedure of tissue culture can be applied profitably to physiological investigation. It is probable that the study of pure strains of cells and of their interactions with the humors of the organism will play as important a part in the progress of physiology as the use of pure cultures of microörganisms in the development of bacteriology.

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INSULIN

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The hypothesis that the anti-diabetic function of the pancreas is dependent upon the internal secretion of a hormone has been accepted by the majority of physiologists ever since 1889 when Mering and Minkowski performed their classical experiments. The evidence has been largely of an indirect nature, however, since no one could succeed with certainty in detecting this hormone in extracts of the gland.

Several observers, particularly Zuelzer (1907), Knowlton and Starling (1912), Clarke (1917), Murlin (1913), Kleiner (1919), E. L. Scott (1911) and Paulesco (1921), succeeded occasionally in demonstrating the existence of substances capable either of diminishing one or other of the symptoms of diabetes in laboratory animals, or of increasing the rate of consumption of sugar by the perfused heart, but the observations were never carried far enough to carry conviction that a hormone capable of controlling all the symptoms of diabetes had been demonstrated. Among the most important of these investigations were those of Clarke who, in 1916, succeeded in showing that the consumption of sugar by the excised mammalian heart is definitely increased when the Locke's solution with which it is perfused is also passed through the blood vessels of the pancreas. He also showed by perfusion through the pancreas alone, that the sugar in the Locke's solution undergoes some change in its stereoisomeric structure as revealed by polariscopic examination, although its reducing properties are unaffected.

There could be little doubt of the existence of the hormone;[1] the problem was to obtain it in extracts of the gland. Assuming that failure to do this must depend on the fact that the hormone is destroyed by the proteolytic enzymes, also necessarily present in the extracts, F. G. Banting undertook to study the effect on diabetic (depancreatized) dogs of extracts prepared from glands in which, by previous ligation of

[1] The name insulin was adopted for the pancreatic extracts without our knowing that it had previously been suggested by Sir E. Sharpey Schafer in 1916 for the then hypothetical hormone.

the ducts, the acinar cells had been caused to degenerate to a much greater extent than the insular, a fact which had been established by several previous workers (Schultz (1900), Ssobolew (1900), W. G. MacCallum (1909), R. R. Bensley (1911), etc.). With the assistance of C. H. Best, Banting (1922) demonstrated in my laboratory that these extracts, when given intravenously or subcutaneously, cause reduction in the sugar of the blood and diminution in the excretion of sugar in the urine. They subsequently found that similar results could be obtained by using, as Zuelzer and E. L. Scott had done before, extracts prepared from pancreas (of the ox) by means of alcohol. Not only were the hyperglycemia and glycosuria greatly reduced in degree by these extracts but the animal improved strikingly in its general condition so long as the extracts were administered, one animal living for seventy days, when it was chloroformed for purposes of investigation. With the collaboration of J. B. Collip (1922), the extracts were now fractionated by alcohol so as to rid them of materials which rendered them unsuitable for continued use on man, because of local irritation, and more extended investigation was made of their effects on diabetic animals in the laboratory, and on diabetic patients. In this work W. R. Campbell and A. A. Fletcher (1922) participated and it was soon possible to show that every one of the symptoms of the disease could be completely removed by use of the extracts.

These brief introductory remarks will serve to bring us to the part of the investigations on insulin with which the present review is more particularly concerned, namely, its effects on normal animals and on animals rendered hyperglycemic by other means than pancreatectomy, and the possible physiological mechanism of its action. The remedial effects of insulin in diabetes have recently been sufficiently reviewed in other places to make it unnecessary here to allude to them further than to state that both glycogen formation and increase in the respiratory quotient are marked effects when insulin is given along with sugar to diabetic animals or patients. The stimulation of these two processes in the diabetic organism satisfactorily accounts for the reduction in the percentage of blood sugar; and the first question which it was considered should be investigated after these facts had been established, was whether insulin can prevent the development of the hyperglycemia which follows various experimental procedures. As a first step in this part of the investigation insulin was injected by J. B. Collip into normal rabbits, with the result that the blood sugar became reduced, thus affording a ready test object for measuring the relative

potency of the various precipitates which separated out in the purification of the extracts. A full description of the exact behavior of the blood sugar under these conditions and of the curious symptoms which supervene when the hypoglycemia reaches a certain degree will be reviewed later in this article, meanwhile we will consider the effect of insulin on the blood sugar and the glycogen reserves in the various forms of experimental hyperglycemia.

For convenience these may be divided into three main groups: nervous, asphyxial and toxic.

NERVOUS FORMS OF HYPERGLYCEMIA AND GLYCOSURIA. This group includes the well-known piqûre experiment of Claude Bernard and also stimulation of the sympathetic (thoracic autonomic) nerve supply of the liver, either electrically or by the injection of epinephrin. The intensity and duration of the hyperglycemia depends very largely on the amount of glycogen stored in the liver, and it is greatly reduced, if not entirely absent, when rabbits from which glycogen has been caused to disappear by starvation or other means, are used for the experiments.

In figure 1 are given curves showing the behavior of the blood sugar in 5 well-fed rabbits on which piqûre was performed, and in 3 on which it was performed during the hypoglycemia produced by insulin. It is evident that the insulin markedly reduces, if it does not entirely prevent the rise in sugar, and it is significant to note that in two of the animals receiving insulin the glycogen content of the liver was decidedly higher than in those not receiving it (4.4 and 2.64 per cent as compared with 0.59 and 2.0 per cent.)

Greatest attention has been paid to the antagonistic effects of epinephrin and insulin, and for two main reasons. First, because the comparison offers a method for the physiological assay of insulin and secondly, because it has often been supposed that epinephrin may play some rôle in the causation of other varieties of transitory hyperglycemia, if not indeed in the more permanent form occurring in diabetes.

In the earlier observations in which it was shown that insulin may prevent the hyperglycemia caused by epinephrin (Banting, Best, Collip, Macleod and Noble) (1922) it was not attempted to obtain precise data on the quantitative relationship between the effects of the two hormones on the blood sugar. More recently, in collaboration with G. S. Eadie (1923), this has been done with a view to the possibility that a method for the assay of insulin might be elaborated.

To secure approximate uniformity in the glycogen content of the livers of the observed animals (rabbits), solid food was withheld for

a period of 24 hours. Different animals were then injected subcutaneously with varying amounts of the same preparation of insulin and with equal quantities of epinephrin (1 in 1000 adrenalin chloride). The epinephrin was injected in a little over an hour after the insulin since, from other observations it appears that the hypoglycemic effects of insulin are fully developed at this time. Determination of the blood sugar at regular intervals gave the results shown in table 1.

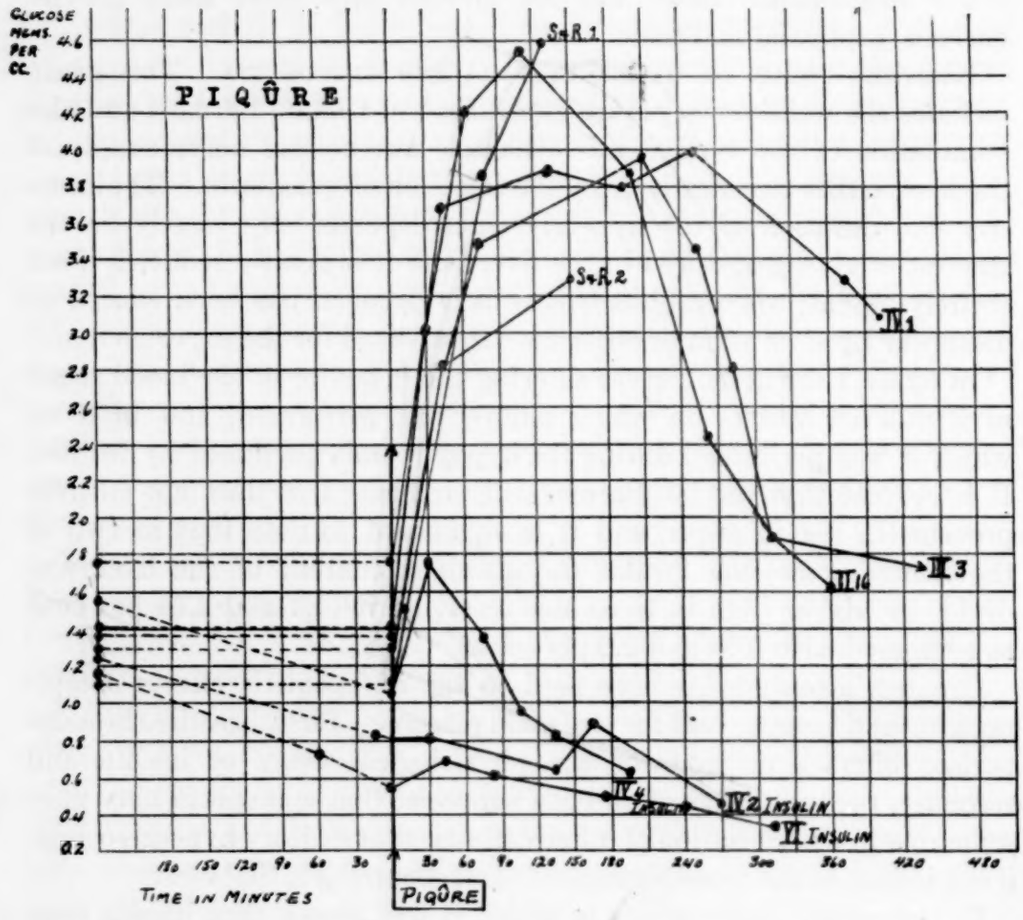

Fig. 1. Behavior of the blood-sugar in well-fed rabbits following puncture of the floor of the 4th ventricle. In the curves marked *Insulin* this was performed on animals that had been injected with this hormone about 1½ hours previously.

In this table the extent of the hypoglycemia caused by varying doses of insulin is shown in the 4th column (marked "Fall") and in those which follow, the extent to which the blood sugar in each case rose at regular intervals following epinephrin. With the largest dose of insulin given

(2 cc. per kgm.) the blood sugar did not rise at all, and it rose to the greatest extent when the dose of insulin was smallest (e.g., with 0.3 and 0.5 cc.). With intermediate quantities of insulin it is seen that the rise was roughly proportional to the dose of insulin. By plotting the rise in blood sugar in milligrams per 100 cc. at any one of the periods of observation (2 hours, for example) against the dose of insulin, the points fall along a curved line, as shown in figure 2.[2] This curve may therefore be used for determining the dose of insulin by finding through how many milligrams per 100 cc. of blood the sugar increases in 2 hours after 1 cc. of adrenalin chloride injected $1\frac{1}{4}$ hours after the insulin.

TABLE 1

DOSE INSULIN PER KGM.	BLOOD SUGAR, MGM. PER 100 CC.								
	Before insulin	Before epinephrin	After epinephrin						
			Fall	½ hour	Rise	1 hour	Rise	2 hours	Rise
1.2	104	5	99	20	15	41	36	38	33
0.7	112	17	95	30	13	58	41	91	74
0.8	107	57	50	92	35	149	92	234	177
0.3	92	59	33	114	55	210	151	287	228
0.5		92		148	56	186	94	279	187
1.4	100	66	34	111	45	134	68	149	83
1.2	106	57	49	77	20			79	22
1.6	111	69	42	48	Fall			53	Fall
2.0	121	28	93					45	17
2.0	125	38	87	42	4	43	5	54	16
1.8	122	46	76	76	30	104	58	159	113

The chief interest of the results, however, rests in the striking antagonism which they demonstrate in the glycemic effects of the two hormones, and the question arises as to the mechanism which is involved.

There can be no doubt that it is mainly by stimulation of glycogenolysis in the liver that the extra blood sugar is derived following injection of epinephrin. Does insulin owe its antagonistic action, then, to its preventing this glycogenolysis, or does it act by causing the sugar, immediately it is produced, to be removed from the blood either by oxidation or by conversion into some non-saccharine substance in the tissues? If we were to base our reply to this question on the effects of insulin on depancreatized animals we should say that insulin both prevents glycogenolysis and encourages oxidation of glucose, but we may not do so, for it does not necessarily follow that what occurs in the diabetic organism will also occur in the normal. The conditions are fundamentally

[2] The equation of this curve is $d.(r)^a = K$ where $a = 0.62$ and $K = 10^{1.08}$.

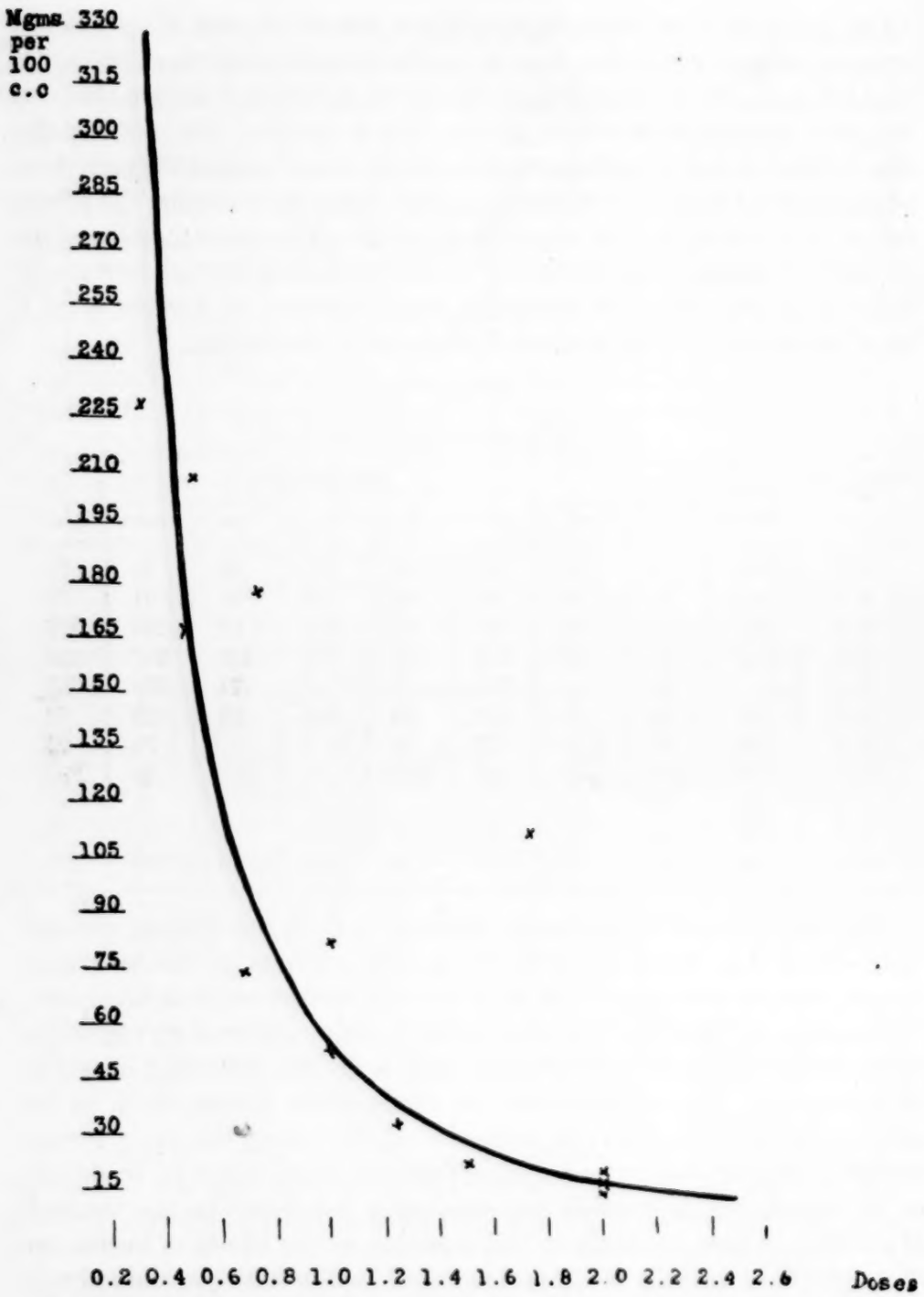

Fig. 2. Curve to show the relationship of the effects of insulin and epinephrin on the blood-sugar of the well-fed rabbits. Equal quantities of epinephrin were injected in all cases in 1½ hours after the doses of insulin indicated in the abscissa. It will be observed that small doses have relatively a much greater influence in preventing the development of hyperglycemia than larger ones.

different; in the normal animal there is present at all times an adequate supply of insulin to meet every requirement for this hormone, so that the injection of more from without induces an abnormal state. In the diabetic, on the other hand, the abnormal state exists, not after, but before the injection of insulin which, therefore, merely allows the metabolic machine to run smoothly again. As a matter of fact, as is discussed

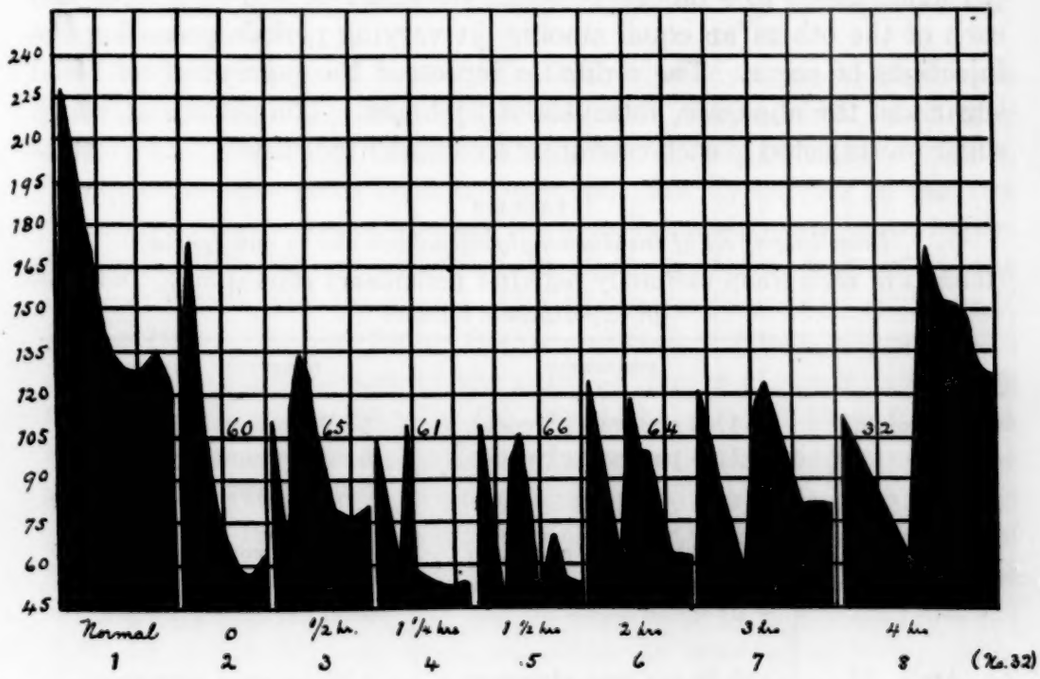

Fig. 3. Curves of blood-sugar showing the effects on a series of starved rabbits of the injection of 2 grams of glucose per kgm. body weight, with and without insulin. The abscissae represent periods of 2½ hours each, the ordinates the percentage of blood-sugar. In the first curve marked normal, no insulin was injected, in those which follow, insulin was injected either along with the sugar, as in curve 2, or preceding the sugar by the intervals indicated in each curve in those which follow. It will be observed that the maximal effect of insulin in preventing exogenous hyperglycemia is developed in from 1¼ to 1½ hours after the injection of insulin.

fully elsewhere (p. 50), when insulin is given along with sugar to glycogen-free rabbits it retards the rate at which glycogen is deposited and when it is given to glycogen-rich animals it causes the glycogen to become less (i.e., encourages, instead of retarding glycogenolysis). Its effect on the combustion of sugar, as revealed by examination of R.Q., is distinctly to increase it, though not to such an extent as to make it likely that this is the main cause for the disappearance of sugar.

We must, therefore, seek for other types of evidence, and the following facts are important: 1, insulin greatly diminishes the degree and duration of the hyperglycemia caused by injections of glucose; 2, it retards the rate of hepatic glycogenolysis due to epinephrin.

The evidence for the first of these statements is given in figure 3 in which are shown the effects of equal injections of glucose (2 grams per kgm.) given to 8 rabbits of which the first received no insulin and each of the others an equal amount at varying periods preceding the injections of sugar. The ordinates represent the percentage of blood sugar and the abscissae, intervals of 2½ hours. The periods at which sugar was injected in each case are stated under the curves. The results

TABLE 2

Retarding effect of insulin on glycogenolysis due to epinephrin

(Rabbits of each group uniformly fed after preliminary starvation.) Duration of experiments 8 hours

	EPINEPHRIN	EPINEPHRIN + INSULIN
March 29	1.40 per cent glycogen	8.12 per cent glycogen
	1.96 per cent glycogen	6.40 per cent glycogen C
		3.64 per cent glycogen C
April 12	8.56 per cent glycogen	12.24 per cent glycogen
	6.60 per cent glycogen	1.60 per cent glycogen C
		5.60 per cent glycogen
April 19	2.30 per cent glycogen	3.34 per cent glycogen
	1.10 per cent glycogen	10.88 per cent glycogen
	3.10 per cent glycogen	

C indicates animal had convulsions.

show that insulin affects both the extent and the duration of the rise in blood sugar, the maximal effect on the former being obtained when the glucose is injected in a little over an hour following the insulin. When insulin and sugar are given simultaneously (2nd curve) it is the duration of the hyperglycemia, rather than its extent, that is affected. After about 2 hours the effect of the insulin begins to weaken and it almost disappears in 4 hours. A method of assay based on these results has been tried but without much success. Their importance in connection with the question at present under consideration is however obvious, for they show that insulin accelerates the rate of disappearance of sugar from the blood. Evidence that insulin also retards the rate of glyco-

genolysis due to epinephrin is given in the following results obtained with E. C. Noble:

From these results we must conclude that in preventing the hyperglycemia caused by epinephrin insulin acts both by inhibiting hepatic glycogenolysis and by accelerating the disappearance of blood sugar into the tissues. This would seem to indicate that a diabetic state is set up in the body by epinephrin which is comparable with that following pancreatectomy.

It has been supposed by some that the hyperglycemia which follows pancreatectomy is due to uncontrolled action of epinephrin, or of some other adrenal function, because of removal of the pancreatic hormone. Various attempts have been made to put this hypothesis to the test of experiment, by seeing whether the symptoms of glycosuria or hyperglycemia diminish or disappear in depancreatized dogs when the adrenal glands are removed. The earlier experiments of Zuelzer, Mayer (1908), Frouin (1908), Hédon and Giraud (1920), did not yield conclusive results because of the extreme degree of shock into which the animals were brought by the double operation. During the few hours that the animals survived, the glycosuria diminished because of the very great curtailment in urine formation, and the percentage of blood sugar became much less, as it apparently always does in conditions of extreme shock. To test the hypothesis properly it is necessary to devise means by which the animals can be kept alive in a tolerably normal state for a few days, at least, following the removal of both glands. Even in the very carefully conducted experiments of Hédon and Giraud, however, this could not be achieved. These workers extirpated the adrenals in dogs from which all of the pancreas had previously been removed except for a graft (the uncinate process) which had been transplanted under the skin of the abdomen. On removal of the graft, immediately following the adrenalectomy, it was found that the blood sugar only rose slightly and that it fell below the normal as the animal gradually became moribund, death occurring after a period of some 7 hours.

It is now recognized that the quickly fatal issue of double adrenalectomy depends mainly on the removal of the cortex rather than the medulla of the gland, which latter is however the source of epinephrin. Taking advantage of this fact Stewart and Rogoff (1923) have repeated these experiments by excising one adrenal and removing the other from the possibility of producing epinephrin by completely cutting all its nerves and also destroying the medulla by means of a curette. After

this operation the animals live in tolerably normal condition, although they lose in weight. In one of them, 51 days after the operation, the blood sugar being 0.096 per cent, the pancreas was removed, with the result that marked hyperglycemia developed (0.198 per cent in 5½ hours, 0.286 per cent in 2 days, and 0.278 per cent in 6 days following the pancreatectomy) accompanied by glycosuria. The animal lived for a week after the pancreatectomy, consuming daily large quantities of meat. In another experiment yielding similar results, the remaining (curetted) adrenal was excised 5 days after the pancreatectomy but even in 18½ hours after the operation the blood sugar was still 0.288 per cent. After this it began to fall reaching 0.08 per cent in 48 hours after the adrenalectomy. It died of collapse in 67 hours when the blood sugar was 0.054. As would be expected the excretion of urine became greatly curtailed as collapse developed and the percentage of sugar in it much reduced.

These experiments show that there is not a particle of evidence for the so-called adrenal hypothesis of pancreatic diabetes, and, as has been pointed out by Stewart and Rogoff, one of the best of Hédon and Giraud's experiments really supports this conclusion, although these authors appear still to hold to the view that a functional relation exists between the pancreas and the adrenals for the metabolism of sugar. In the experiments referred to the adrenals were removed from a previously depancreatized dog without causing *any* reduction in blood sugar after 8 hours, and only a moderate reduction (from 0.3 to 0.22) in 23 hours. It is impossible to interpret these results in terms of the adrenal hypothesis of pancreatic diabetes. In view of the antagonism which we have seen exists in such striking fashion between epinephrin and insulin, with regard to their effects both on hyperglycemia and glycogenolysis, it seemed of interest to see whether insulin has the same effect on hyperglycemia in animals that are simply depancreatized, as on those that are both depancreatized and adrenalectomized. Stewart and Rogoff have made this comparison without finding that there is any difference. Had it been found that insulin acted much more effectively on the animals still retaining the adrenals, the result would have given support to the adrenal hypothesis. These workers have also shown that insulin acts on rabbits which have survived complete removal of the adrenal glands in exactly the same manner as on normal rabbits and also that the amount of epinephrin contained in the blood of the adrenal veins is unchanged by injections of insulin.

These experiments are referred to in some detail because they are the only ones so far attempted to throw light on the nature of the con-

dition which becomes developed in the body following pancreatectomy, and which is the immediate cause of the diabetic state. We know that insulin opposes this influence, but we know nothing of what it is. Even if it could be shown that insulin is required to change glucose into some active form, and thus prepare it for oxidation or polymerization, this would not explain why in pancreatic diabetes the production of glucose out of protein and fat becomes so excessive.

ASPHYXIAL FORMS OF HYPERGLYCEMIA. The asphyxial hyperglycemias that are best known are those due to mechanical asphyxia, and to anoxemia caused by carbon monoxide. It is possible also that the hyperglycemia caused by general anesthetics, such as ether, may belong in part at least to this class.

Insulin has the property of preventing hyperglycemia in each of these conditions. In mechanical asphyxia (brought about in well-fed rabbits by almost completely obstructing the air passages until slowing of the heart occurs) the rise in blood sugar is even more prompt and extensive than it is following epinephrin or piqûre, but only a slight rise occurs in animals that are under the influence of insulin. This method has the drawbacks, first, that the asphyxial condition can be maintained with safety for only a short period of time (15 minutes), and second, that it is impossible to say whether hyperglycemia is due to the accumulation of carbon dioxide in the blood, (which might excite increased glycogenolysis because of increase in H-ion concentration) or to lack of oxygen (anoxemia). It is therefore of interest that insulin also prevents hyperglycemia from developing in the more prolonged condition of anoxemia brought about by causing the animal to breathe air containing about 0.8 per cent of carbon monoxide. In a well-fed rabbit exposed to such an atmosphere for about 45 minutes the blood sugar immediately after the exposure was found to be about 0.300 per cent and to continue rising for about an hour, after which it slowly declined. When the same rabbit a week later was given insulin and again subjected, for the same length of time, to 0.8 per cent carbon monoxide, the blood sugar remained absolutely unchanged. Similar observations on other animals made it certain that insulin can entirely prevent the development of hyperglycemia in gassed animals.

TOXIC FORMS OF HYPERGLYCEMIA. The effect of insulin on ether hyperglycemia calls for some attention. When this anesthetic is administered by the usual methods the blood sugar in well-fed rabbits rises very quickly to a high level and, anesthesia being maintained, usually remains unchanged for a considerable time (see fig. 4). According to Fujii (1921) the degree of the hyperglycemia is proportional to the intensity of the

etherization. In dogs the initial rise in blood sugar is also sharp, but not, as a rule, so pronounced. Considerable oscillations, however, may occur even though the degree of anesthesia is kept as constant as

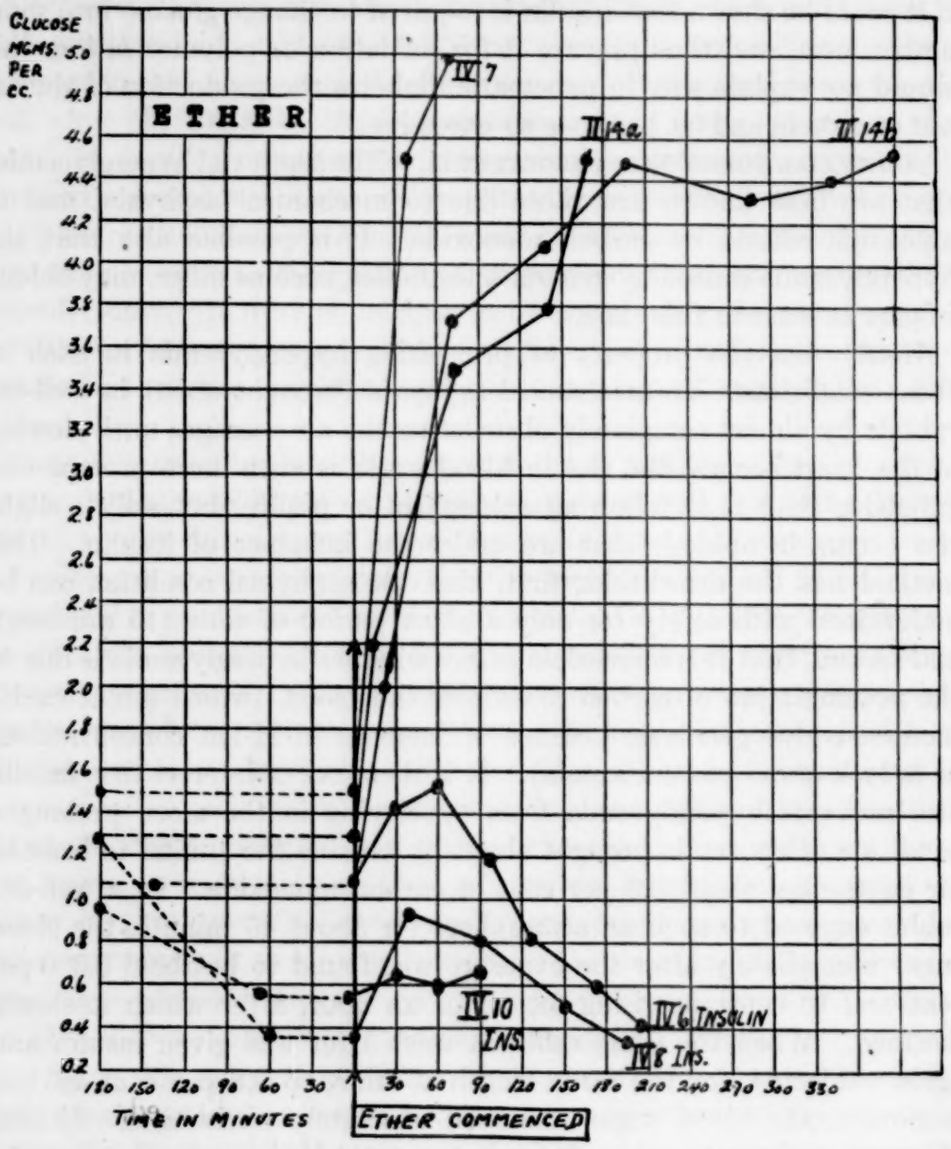

Fig. 4. Blood-sugar curve of rabbits showing the effect of insulin in preventing ether hyperglycemia.

possible and it is important to remember, in connection with experimental work on anesthetized animals, that the blood sugar may fall considerably when the blood pressure, for any reason, becomes subnormal and also that it rises when anything occurs to interfere with respiration (anoxemia).

When ether is given to rabbits during the hypoglycemia due to insulin the blood sugar only rises to a slight extent, if at all (see fig. 4) and when it is given to rabbits in which ether hyperglycemia is already present it rapidly causes the blood sugar to fall. When insulin is given to dogs in which the hyperglycemia due to ether is already established more is required to bring the blood sugar to within the normal limits than when the animals are given insulin prior to the administration of ether. This observation corresponds to the clinical experience that insulin is of much greater value in diminishing the risks of surgical operations on diabetic patients when it is given for some time prior to administration of anesthetics than when given only at the time of operation. This may partly be because any, even a transient, degree of hyperglycemia increases the surgical risks, but another and probably a more important reason is that the preliminary treatment with insulin causes glycogen to be deposited in the liver.

All of the foregoing forms of hyperglycemia have been shown by Stewart and Rogoff to be unassociated with any increase in the internal secretion of epinephrin from the adrenals. On the other hand, it has been found by these workers that the hyperglycemia which becomes developed (in the cat, dog and rabbit) as a result of injections of morphine are in some way associated with the adrenal glands, the evidence being: *1*, it does not occur in animals in which one adrenal is excised and the other, both denervated and its medulla destroyed by curetting; *2*, an increased secretion of epinephrin into the blood of the adrenal veins can be shown to occur after injections of morphine.

It therefore became of interest to see whether insulin would prevent morphine hyperglycemia and Stewart and Rogoff (1923) have found this to be the case. Incidentally they have also found that insulin does not affect the general symptoms of morphine poisoning although it prevents the rise in body temperature which, in cats, otherwise follows administration of this drug. They were unable to show that insulin has any effect on the output of epinephrin from the adrenal glands.

Two other substances having effects on carbohydrate metabolism are pituitrin and phloridzin, but neither of them acts, primarily at least, by causing increased glycogenolysis. They do not therefore belong to the same category as those we have been considering (epinephrin and the anesthetics).

Pituitrin, a hormone derived from the infundibular portion of the pituitary gland, given intravenously, usually causes hyperglycemia in normal animals, although sometimes it causes no change or even slight

hypoglycemia; when it is given along with epinephrin, however, it inhibits the development of the hyperglycemia. This arouses interest as to what effect pituitrin will have when given along with insulin, and Burn (1923) has found that it may either entirely prevent the development of hypoglycemia or make it less pronounced, according to the dose of pituitrin.

TABLE 3

Effect of insulin on R.Q. in phloridzin diabetes

DATE	CONDITION	R.Q.
July 8, 1922	P.	0.71
	(10.05) 40 grams sucrose	0.69
July 10		0.67
	(12.00) 40 grams sucrose	0.66
July 11	(11.05) insulin	
	(12.50) 40 grams sucrose	0.91
July 12		0.66
	(1.00) 40 grams sucrose	0.82
Sept. 8		0.65
	(12.10) 40 grams sucrose	0.70
Sept. 9		0.65
	(10.50) insulin	0.72
	(11.50) 40 grams sucrose	0.86
Sept. 11		0.74
	(1.15) 40 grams sucrose	0.80
Sept. 13		0.73
	(10.50) insulin	
	(11.50) 40 grams sucrose	0.89
Sept. 14		0.72
	(12.55) 40 grams sucrose	0.87

With regard to phloridzin little can be said here except that it was found early in the investigations that insulin is capable of causing the respiratory quotient to rise when it is given along with sugar to animals that are under the influence of this drug (S. U. Page). This is shown in table 3.

THE EFFECT OF INSULIN ON THE BLOOD SUGAR OF NORMAL ANIMALS. Since insulin can lower the blood sugar in all forms of hyperglycemia, whether due to removal of the pancreas or to increased glycogenolysis or to injections of glucose, it is to be expected that it will also lower the blood sugar in normal animals.

As has already been stated this was found to be the case at an early stage in the investigations, thus providing a ready means for measuring the strength of the various preparations of insulin. It was also observed that peculiar symptoms developed some time after the insulin was injected. In their general character these symptoms in the rabbit were seen to be comparable with those which had been observed by Mann and Magath (1921) to develop in dogs when the blood sugar has fallen to about 0.045 per cent as a result of removal of the liver, and which these workers definitely showed to be dependent on the hypoglycemia, since they were removed, temporarily at least, by injections of glucose. By comparing the incidence of the symptoms with the percentage of blood sugar in rabbits injected with insulin the same relationship was found to hold, and by injecting glucose solutions subcutaneously the symptoms were immediately removed, the animals returning permanently to a normal condition.

It will be convenient to consider the further investigations of the interesting problems which these results point to under the following headings:

1. The nature of the symptoms and the physiological mechanism of their production.
2. The relationship of the symptoms to the blood-sugar level and their removal by raising this.
3. The physiological factors which influence the rates of decline and of recovery of the blood sugar.

The nature of the symptoms due to insulin.[3] These have been observed to occur in all types of animals. In rabbits, in which animals the symptoms were first observed, they consist of violent convulsive seizures in which the animal throws itself over sideways, usually first in one direction, then in the other, with the head retracted and the hind limbs in an extended position. The condition is not unlike that caused by strychnine or by acute asphyxia, except that certain groups of muscles are less affected by the convulsions than others. After a period, which varies from 30 seconds to a minute or so, the convulsions cease and the animal

[3] This description of the symptoms is taken from my lecture before the Eleventh International Congress of Physiology at Edinburgh, July, 1923.

lies on its side apparently in an unconscious state, with rapid, shallow breathing. The exact condition of the animal at this stage may, however, vary considerably, well-fed and therefore glycogen-rich animals often behaving in apparently normal fashion between the convulsive seizures, which are often brought on by attempts to move. The convulsions are more violent in well-fed as compared with starved animals, and they seem to be in some way related to the source of the insulin, being particularly severe with that obtained from skate. Rabbits seem to acquire a certain degree of immunity to the convulsions for these become less marked in animals that have been frequently injected with insulin. After a varying period the comatose stage is followed by another convulsive seizure, and these phases may continue alternately for an hour or more, the convulsions becoming feebler and feebler and the rectal temperature falling, until at last the animal dies of respiratory failure. After death *rigor mortis* sets in at once. The arterial blood is venous in color, and it clots very quickly. Preceding the onset of typical convulsions there are usually premonitory symptoms of hyperexcitability and evidence of hunger; sometimes, however, paralysis of the extremities is the first symptom.

The symptoms vary somewhat in other animals. In the dog the first signs are usually very rapid breathing, restlessness and general hypersensitivity, muscular twitching then becomes evident and the sphincters may relax. Barking is often a prominent symptom, and there may be frothing at the mouth. At this stage, or as the first symptom, convulsions not unlike those seen in the rabbit may supervene, and between them the animal lies on its side showing violent twitchings of the musculature, almost a tetany. It is evidently unconscious. The rates of breathing and of the pulse increase. Inspiration is usually short and jerky, and inspiratory tetanus not infrequent, so that artificial respiration may have to be applied. Attempts to get on its feet are often the cause for convulsive seizures and, during recovery, the muscles of the extremities, particularly the anterior, are seen to have entirely lost their power of coördinate action. In etherized dogs even massive doses of insulin have no immediate effect on the blood pressure.

In the cat the symptoms are like those in the dog, profuse salivation, mewing, and relaxation of the sphincters being especially marked.

In the mouse kept at room temperature, A. Krogh observed that insulin may only cause the animal to become weak, and its temperature to fall to a very low level. If it be kept in an incubator at about 28°C., however, characteristic symptoms supervene—convulsions and coma,

preceded by paralysis of the legs. This influence of body temperature is particularly interesting, since it probably explains the apparent immunity of frogs even to massive doses of insulin. We failed to note any symptoms in three days after injecting insulin, but A. Krogh has since found that they occur in a day or so later, a fact which we have confirmed.

In man, in whom the subjective symptoms can be interpreted and cardio-vascular changes more accurately observed, the picture is different. When the blood-sugar reaches about 0.075 per cent, the patient experiences extreme hunger and a sense of fatigue. He usually becomes anxious and may lose his emotional control. Actual tremor of the musculature is rarely seen, but there is a definite sense of tremulousness and some incoördination for fine movements. "Vasomotor phenomena are common; pallor or flushing, sometimes one after the other; a sense of heat, of chilliness, almost always a profuse sweat" (Banting, Campbell and Fletcher (1923). At lower levels of blood sugar, acute mental distress, mental disturbances, delirium, and finally coma, with loss of the deep reflexes, supervene.

The susceptibility toward the development of symptoms varies in different animals of the same breed, sex, age and weight. One animal, for example, was observed by us to remain free from symptoms with doses of insulin that were several times larger than those causing marked symptoms and hypoglycemia in others. The occurrence of such refractory animals makes it clear that the incidence of symptoms cannot be used for purposes of assay of insulin unless very large numbers of animals are used.

The physiological mechanism involved in the production of the hypoglycemic symptoms. In their general nature the convulsions point to a violent stimulation of the higher nerve centers controlling muscular action, with involvement of those of respiration, salivation and phonation. The convulsive movements, by themselves, are very similar to those which Barany has shown can be brought on in rabbits by spinning them, and which are undoubtedly due to abnormal stimulation set up by labyrinthine disturbances of the centers which coördinate muscular activity. These symptoms produced by spinning in normal animals are, however, only of short duration and they are accompanied by nystagmus whereas those following insulin are usually much more prolonged and are unaccompanied by nystagmus. When animals showing premonitory symptoms as a result of insulin are spun their susceptibility to the spinning reactions is not increased.

It would appear, therefore, that the stimulus which is set up by the lowering of blood sugar acts in the first instance on certain of the nerve centers in the region of the pons and medulla. That the lower muscular neurones are not primarily affected has been shown by Olmsted and Logan (1923), who found that no symptoms supervene in spinal cats even when, by insulin injections, the blood sugar has been caused to fall far below the usual convulsive level. In decerebrate animals, on the other hand, typical symptoms supervene provided the pituitary glands have been removed. Unless this is done the blood sugar does not become sufficiently lowered, even by massive doses of insulin, probably because of the antagonistic action of pituitrin (see p. 34).

These facts are illustrated in the following results (table 4) obtained by Olmsted and Logan.

TABLE 4

Blood sugar percentages at hourly intervals

	1	2	3	4	5	6	7	8
Decerebrate, pituitary intact	0.230	0.270	0.300*	0.170	0.160			
Decerebrate, pituitary removed	0.230	0.212*	0.124	0.080	0.058*	0.052		
Decapitate, insulin at start (no convulsions)			0.028	0.040	0.070	0.068	0.016	0.028
Decerebrate, pituitary removed insulin at start			0.048 C	0.026 C	0.040 C			

* Insulin injected.
C = Convulsions.

There is much in the general nature of the symptoms to suggest that a failure of oxidative processes in the nerve cells is their immediate cause. Thus, as Olmsted and Logan (1923) have pointed out, the behavior of a cat, or a rabbit, after clamping the trachea is very like that of one injected with insulin. In both cases the animals run about blindly bumping into objects in their path and then falling on the side, exhibiting running movements with the fore legs, the hind legs being tightly flexed on the abdomen. In some cases, also, asphyxiated animals show the rolling movements that are so common after insulin. This lack of oxygen in the nerve cells may result either from a failure in the supply carried to them by the blood or to the development within their protoplasm of some condition which renders them incapable of utiliz-

ing the oxygen with which they are supplied. Some support is given to the former possibility by the fact that the arterial blood, at the time the symptoms supervene following insulin, is decidedly venous in color and clots almost immediately it is withdrawn. On the other hand the incidence of symptoms does not appear to be affected by placing the animals in an atmosphere of pure oxygen and there is no doubt, in dogs at least, that the consumption of oxygen becomes markedly increased as the symptoms supervene. These facts would seem to show that the inspired oxygen is locked away in some form in the tissues so that its tension in the blood supply in the brain is inadequate.

The relationship of the symptoms to the blood-sugar level. In the earlier experiments the blood sugar was usually found to be about 0.045 per cent when convulsions supervened. Thus, out of a total of 123 rabbits receiving insulin, convulsions were observed in 26 cases and the maximum percentage of blood sugar at which they occurred was 0.047 (except in one case where it was reported as 0.067) and the minimum percentage at which no convulsions were observed was 0.037. These animals had been fed up to the time of injection, so that considerable quantities of glycogen must have been present in the liver and muscles. Since the amount of glycogen may vary considerably in different animals, although they be fed alike, and this, as we shall see later, has an influence on the behavior of the blood sugar following insulin, it has been the practice, during more recent work in which insulin has been assayed on this basis, to use animals that have been starved for 24 hours preceding the injection. Under these conditions convulsions often fail to occur until the blood sugar is considerably below 0.040 per cent, this being particularly the case when a large dose of insulin is given, so that the initial rapid descent in the blood sugar is continued longer than usual (see p. 42). Thus, out of a total of 335 rabbits used by Mr. Orr for purposes of the assay of insulin, no symptoms are recorded in 64 in which one or more of the blood sugars, examined in 1½, 3 and 5 hours after the injection, were decidedly below 0.040 per cent. The discrepancy between the results of the two groups of observations is probably more apparent than real. There is no doubt that convulsions are almost certain to occur if the blood sugar be kept for a sufficient length of time at about 0.045. This is apparently what occurs in glycogen-rich rabbits in which the blood sugar falls rapidly following insulin until a certain critical level is reached, when its further decline is counteracted by mobilization of sugar from the glycogen stores. This maintains it at or about a level at which unphysiological (toxic) con-

ditions responsible for the development of symptoms are slowly developed. On the other hand in starved animals the blood sugar, through its failure to be replaced, continues its rapid decline and passes below the usual convulsive level before there has been time for the development of the toxic conditions.

In other animals, the relationship between the blood sugar level and the appearance of symptoms is much the same as in rabbits. In the dog, and still more so in man, however, the premonitory symptoms are much more easily detected and they can be observed to make their appearance while the blood sugar is still considerably above the convulsive level. Thus in the dog, respiratory symptoms and evidence of hyperexcitability and even muscular tremors and incoördination are common when the blood sugar is about 0.060 per cent and in man hunger pains and other subjective symptoms have been observed with the blood sugar at 0.075 per cent.

The removal of the symptoms by injection of glucose. The association of hypoglycemia with characteristic symptoms, as has already been pointed out, recalls a similar relationship observed by Mann and Magath to exist in dogs from which the liver has been removed. As in these experiments also they can be antidoted by restoring the lost glucose to the blood. This can best be accomplished by injecting 2 grams glucose per kilo body weight in isotonic solution, subcutaneously, the exact time necessary for recovery depending on the extent to which the symptoms have been allowed to continue. When the glucose is given immediately the first definite symptoms set in, recovery occurs within 3 to 5 minutes but when its administration is delayed until coma, rather than convulsions, has become the prominent symptom a much longer period, one half-hour or more, may be necessary before the animal is restored to a tolerably normal state. The delay under these conditions depends in part on the sluggish state of the circulation and it is interesting to note that the first sign that recovery is setting in is often that convulsive seizures become more frequent and the breathing, from being either very rapid and shallow or of a periodic type, becomes slower, deeper and more regular. The rate of recovery also depends on the size of the animal (2 grams glucose per kilo body weight being the amount given in each case); in large dogs, for example, even when the injections of glucose are made immediately after definite symptoms have been noted, it may take several minutes before recovery is complete whereas in mice this occurs within a minute or two. After recovery the animals usually take food greedily and, not infrequently, they then become drowsy,

being perfectly normal in every way after awakening from their sleep. Frequent repetition of the symptoms due to injections at intervals of a few days apart does not affect the general well-being of the animals, indeed they usually increase in weight. As a rule they also become less susceptible to the symptoms so that the blood sugar may be lowered much below the level at which the symptoms previously occurred.

Of all the sugars and sugar derivatives so far investigated, glucose stands alone in its antidoting action (Noble and Macleod, 1923). Mannose, galactose and levulose have a certain value in this regard but the pentoses, xylose and arabinose, are entirely inert, although of course they cause the reducing power of the blood to become increased. Of the disaccharides, cane sugar and lactose are inactive, but maltose causes a certain degree of recovery with frequent relapses, evidently because of its partial inversion by the maltase of the blood. The reducing power of the blood becomes raised after maltose and lactose but not after glycogen, given subcutaneously, neither does this polysaccharide have any effect on the symptoms. Sodium lactate and glycerol have no effect. The remarkable specificity of glucose in removing the symptoms makes it of interest to determine the exact part of the molecule to which its action is due. So far as they go, the foregoing observations would seem to indicate that the aldehyde group of the open chain formula cannot alone be responsible since this is only partially obliterated in maltose, and is entirely free in the pentoses and in galactose and mannose. The inactivity of the monosaccharides other than glucose indicates further that the alcohol (-OH) side chains are not in themselves responsible for the action. Indeed as far as can be judged at present the intact glucose molecule is necessary—not any particular side chains but the molecule as a whole—but further investigations at present under way, in collaboration with J. C. Irvine and P. T. Herring, in which the action of the various methyl glucosides and natural glucosides is being investigated may throw further light on the problem. A similar specificity of glucose was observed by Mann and Magath (1921) in their experiments on dogs after removal of the liver. In animals that have been well fed prior to the injections of insulin the blood sugar, after it has fallen to a certain level, is likely to recover spontaneously, because of mobilization of glucose from the glycogen stores. This mobilization may be greatly accelerated by injections of epinephrin and this may be used in place of glucose injections to antidote the hypoglycemic symptoms. When this method is used in clinical practice, however, it is necessary to be certain that glycogen is present in the liver, which, in previously untreated cases

of severe diabetes, is unlikely to be the case. The blood sugar may also recover, though much more slowly, in starved animals in which only traces of glycogen can be detected and in such cases we must assume that new glucose is produced out of protein or, it may be, out of fat. This offers us, therefore, a possible method for studying the influence of various substances on the process of gluconeogenesis.

The physiological factors which influence the rates of decline and recovery of the blood sugar. When insulin is injected into rabbits the blood sugar almost immediately begins to fall and there is remarkably little differ-

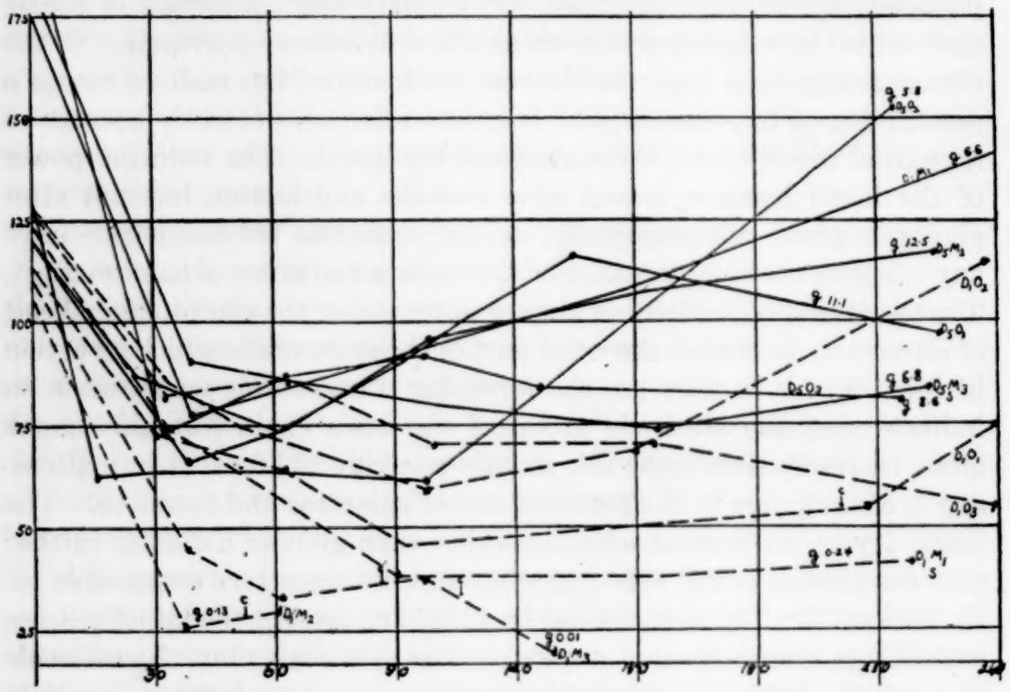

Fig. 5. Abscissae, minutes after injection of insulin. Ordinates, mgm. sugar per 100 cc. blood. Broken lines—starved animals. Unbroken lines—fed animals.

ence, either in the extent of the fall or in the time of onset, according to whether the injection is made intravenously or subcutaneously. Within 10 minutes of the injection a decided lowering of blood sugar is almost always perceptible, although occasionally, especially in previously well-fed animals, a slight increase may be observed in this time. The fall continues almost in a straight line for from 30 to 45 minutes and it is remarkable that, within very wide limits, the steepness of descent is independent of the dose of insulin and also of the nutritional state of the animal. This is shown in the accompanying curves (fig. 5).

A certain degree of parallelism between the dose of insulin and the steepness of the initial fall in blood sugar can be detected when very small amounts are injected but this is not close enough to be of any value for purposes of assay, at least when different animals are used.

The level of blood sugar existing at the time of injection does not affect the rate of the initial fall unless the level be very high, when the descent becomes somewhat more rapid. Neither does the amount of glycogen stored in the liver affect the initial fall although it has a marked influence on the ultimate extent to which the blood sugar subsequently descends.

After the period of initial fall the curve gradually becomes more horizontal but from this point on its behavior is dependent upon several easily determinable factors, the most important of which are: *1*, the dose of insulin; *2*, the amount of glycogen stored in the liver; and *3*, the occurrence of convulsions. The relationship between the extent to which the blood sugar becomes lowered and the dose of insulin, as observed in starved animals, is an interesting one since it follows a logarithmic curve like that which F. N. Allan (1923) has found in the case of glucose equivalents when insulin is given to depancreatized dogs. The reasons for this relationship are partly that when larger doses are given much of the insulin is excreted in the urine, or destroyed, and, partly, that the laws of enzyme action are probably followed. The relationship between dosage and effect in the case of insulin would appear to conform with that for drugs in general (see Murray Lyon, 1923).

The influence of the glycogen stores is readily demonstrable when comparisons are made between animals that are either rich or poor in glycogen, but it is to be noted that no relationship between the ultimate extent to which the blood sugar declines and the percentage of glycogen is to be expected when the glycogen reserves are all above a certain minimum level. It is particularly with regard to the rate at which the blood sugar ascends again that the glycogen stores are of importance. With small doses of insulin animals rich in glycogen may show only a transient fall in blood sugar lasting *in toto* only for about an hour, whereas the same dose given to those that are practically glycogen free, may cause a profound and prolonged fall coupled with convulsions. (See fig. 5.) The bearing of these observations on the use of insulin for therapeutic purposes on man is self evident.

The blood sugar often rises perceptibly immediately following a convulsive seizure. This can be demonstrated by comparing blood samples taken immediately the first signs of a convulsion are seen and within a few minutes after it. It is especially marked when well-fed

animals are used and the dose of insulin is just sufficient to bring the sugar to the convulsive level. Even in a-glycogenic animals, however, a certain increase in blood sugar has sometimes been observed to follow a convulsion, the extra sugar in this case being derived probably from protein.

The mechanism of action of insulin. The very rapid fall in blood sugar which sets in almost immediately after injection of insulin suggests that some process occurring in the blood itself must be responsible for it—an increased glycolysis. Were this the case the addition of insulin to blood kept at body temperature outside the body might be expected also to accelerate the rate of disappearance of sugar, but it has been impossible to demonstrate that this occurs (Eadie, Macleod and Noble, 1923). Since it is possible, however, as has been suggested by Cohnheim, that the pancreatic hormone does not in itself cause acceleration of the breakdown of sugar in the animal, but acts in association with an enzyme furnished by the muscles, observations have also been made on the comparative rates of glycolysis in incubated blood withdrawn from normal animals before and at varying periods following the injection of insulin, again without detecting any difference. Neither could insulin be shown to have any effect on the rate at which sugar disappears from a mixture of a sterile saline (Buchner) extract of muscle and glucose, or of one consisting of muscle extract, defibrinated blood and glucose, or of a suspension of leucocytes (sterile pus) and glucose. The conclusion is, that so far as can be judged by estimation of the percentage of glucose by the usual methods (measurement of reducing power), insulin does not influence the rate of glycolysis. It is possible, however, that it might have an influence on some property of glucose other than its power to reduce metallic oxides or picric acid, for example, its stereo-chemical properties, or its power to reduce other substances such as methylene blue or permanganate. With regard to the former of these possibilities, Winter and Smith (1923) have stated that the dextro rotatory power of solutions of glucose becomes diminished when they are acted on by insulin in the presence of an extract of liver. They also state that normal blood after removal of protein, by means of a lengthy and complicated process, rotates polarized light to the right to a less extent than corresponds to the amount of ordinary ($\alpha\,\beta$ glucose), as determined by reduction methods, but gradually reaches this value after the solutions have stood for some time (a day or so). In diabetic blood, on the other hand, the observed rotating power of the blood extracts corresponds to, or is higher than that of $\alpha\ \beta$ glucose, but when

insulin is injected conditions similar to those of normal blood become established. Winter and Smith have suggested that these results may indicate a change from the more stable (α, β) varieties, as which glucose exists in a simple solution, to the less stable variety such as that recently identified by Irvine and his pupils, and styled γ glucose.[4] The relative instability of this newly identified variety of glucose is manifested also by its ability to cause rapid reduction of permanganate solutions in the cold, which α β glucose can only do slowly. Winter and Smith's hypothesis is that glucose must be converted into the less stable variety before it can be used in metabolism, and that insulin is necessary for this conversion. There is as yet no satisfactory evidence to support the hypothesis and Eadie (1923) has so far failed to obtain results with mixtures of liver extract, glucose and insulin that agree with those reported by Winter and Smith. Hewitt and Pryde, previously to the work of Winter and Smith, had stated that solutions of ordinary glucose give polariscopic evidence of the formation of γ glucose when placed in loops of intestine. We have failed to confirm this, so also have Stiven and Waymouth Reid (1923) who have also shown that on theoretical grounds such changes could not be expected to occur in glucose solutions placed in loops of intestine.

In extracts of normal blood prepared exactly according to Winter and Smith's directions it is the case, as Eadie has shown, that polarimetric readings are often obtained which are less dextrorotatory than they should be (as judged from the reducing power) and which slowly become greater on standing, but this, instead of indicating the existence of γ glucose, may depend on the presence, either of glucosides which gradually become hydrolyzed on standing, or of traces of levorotatory substances which become gradually destroyed. Other criticisms of Winter and Smith's results are that the polarimetric readings are extremely small in magnitude and that the increase in dextrorotatory power upon which rests the conclusion that γ glucose is changed into

[4] The differences in stability are believed to depend on the exact manner of linkage of the oxygen atom in the ring form of the formula for glucose, this being between the 1st and 4th (or butylene) carbon atoms in the more stable $\alpha\beta$ forms and between the 1st and 2nd (or ethylene) carbon atoms in the γ form. Since this ethylene form rotates the plane of polarized light to the left its formation can be detected in an equilibrated mixture of the α and β forms, which rotate strongly to the right, by a diminution in the dextro rotatory value without any change in the reducing power of the solution and, since γ glucose is very unstable, by the rotatory value quickly returning to that of an equilibrated mixture of $\alpha\beta$ glucose when the solution is allowed to stand.

α β glucose occupies not a few seconds, as it would be expected to do if a highly labile form of glucose were present, but several days.

Although the evidence so far presented cannot therefore be considered as satisfactory we must not lose sight of the possibility that insulin, acting along with some other agency in the tissues, may cause a change in the glucose molecule rendering it more susceptible to oxidation. Observations by Clarke (1917) and by Ahlgren (1923) lend support to such a view. Clarke, as has already been pointed out, perfused the blood vessels of the pancreas with Locke's solution containing glucose and found that, although the reducing power of the solutions was not affected, a diminution occurred in the dextrorotatory power, and when the solution was then perfused through the excised heart its reducing power became diminished much more rapidly than when fresh Locke's solution, containing the same percentage of glucose, was used. He interpreted this as indicating that the pancreas had yielded to the Locke's solution some hormone which prepared the glucose for combustion by the cardiac muscle. Ahlgren's experiments (1923) were based on the observation that methylene blue becomes reduced to the leuco form when placed *in vacuo* in contact with extract of washed muscle or other tissue and such substances as d-lactic acid, acetaldehyde, acetic acid, etc., but does not become reduced in the presence of α β glucose. The theory of the action is that the oxidases of the tissues (or dehydrogenators) split off active hydrogen from the oxidisable substances (or hydrogen donators) and transfer it to the methylene blue (or hydrogen acceptor). α β glucose cannot act as a hydrogen donator but it becomes capable of doing so, and therefore causes decolorization of the methylene blue, in the presence of insulin and something supplied by the tissues. Entire absence of insulin can only be expected in the tissues of completely diabetic animals and as a matter of fact Ahlgren has found that extracts of the muscles of a depancreatized dog, and of depancreatized frogs, have very little power to decolorize methylene blue in the presence of glucose, but acquire this power when insulin is added. In extracts of tissues from normal animals, even after most careful washing, unremoved traces of insulin were found to cause a certain degree of reduction of the methylene blue, but this increased by adding more insulin. A typical result with extract of thoroughly washed muscle of guinea pig is as follows:

	VACUUM TUBE NO.							
	1	2	3	4	5	6	7	8
1 per cent glucose solution, cc.....	(blank)	0.2	0.2			(blank)		
1 per cent levulose solution, cc....							0.2	0.2
1 per cent insulin, cc.....			0.2	0.2				0.2
$\frac{M}{10}$ potassium lactate, cc.....				0.2	0.2			
Time required for decolorization in minutes.....	72	64	55.5	79	31	73	45	50

Each tube also contained 0.4 cc. 1–1000 methylene blue and 0.2 cc. K_2HPO_4 solution.

It took 72 to 73 minutes for the blue color to disappear in the two blanks, 1 and 6, and when lactic acid was present—to show the effect of a pronounced hydrogen donator—it took only 31 minutes. When glucose and insulin were present, 3, the decolorization was much more rapid than the control, so also was it, although less so, with glucose alone, 2, indicating that the tissue had not been washed free of all traces of insulin. Fructose (and galactose) differs from glucose in acting as a hydrogendonator without the presence of insulin, as is shown by the results 7 and 8. These results are interpreted by Ahlgren as showing that under the influence of insulin the tissues can convert glucose into products which, like lactates, etc., are capable of acting as hydrogen-donators. It is equally possible however that no such actual splitting of the molecule occurs but that a more active form of glucose is formed, or that glucose is converted into one of the hypothetical enolic forms.

In the light of these observations it seems probable that insulin does have some effect on the glucose molecule which renders it more susceptible to oxidation, but what relationship this may bear to the development of hypoglycemia, it is at present impossible to say. In any case, the hypoglycemia is not due to increased glycolysis within the blood itself, and to account for it, which is the important problem at present, two possibilities remain: *a*, that insulin entirely prevents the discharge of sugar into the blood from the liver; *b*, that it accelerates the rate of sugar consumption by the tissues. With regard to the former possibility, the question is whether insulin may act by inhibiting the process of glycogenolysis, the lowering of the blood sugar being then merely the result of a continuance of its normal diffusion into the tissues. It is well known that hypoglycemia quickly develops when the liver is removed from the circulation (Bock and Hofmann, Pavy (1903), Macleod (1909), Mann and Magath (1921)). There are several reasons why this interpretation cannot be accepted:

1. The sugar disappears from the blood after insulin much more rapidly than after removal of the liver.

2. It also disappears after insulin at the same rate in an animal deprived of its liver as in a normal animal (Mann and Magath, 1923).

3. The glycogen stores of the liver become less after insulin instead of remaining constant as they should if the process of glycogenolysis were shut down (see p. 50).

4. The rate of post-mortem glycogenolysis is the same with and without insulin. E. J. Lesser has informed me that insulin does not retard the rate at which glucose is formed in the liver of the frog, indeed it accelerates this process.

By exclusion we are forced to conclude that insulin after entering the blood must pass into the tissues, and set up in them some process which leads to a reduction in the amount of free glucose, thereby creating, as it were, a vacuum for this sugar, with the result that glucose is removed from the blood.

Direct evidence that the sugar disappears into the tissues is furnished by the experiments of Hepburn and Latchford (1922) on the excised mammalian heart of the cat and rabbit perfused at body temperature with Locke's solution containing glucose. Although the rate at which glucose disappears from the perfusion fluid varies with the rate and strength of the heart beat, the average consumption in 11 observations was found to be 0.97 mgm. per gram of heart muscle per hour, the maximum being 1.5 mgm. and the minimum 0.4 mgm. This agrees closely with the figures given by previous workers. When insulin was added to the perfusion fluid—the best method for doing this being to add a drop every 15 minutes—the rate of disappearance of glucose rose on an average about three times: in 8 observations the average was 3.06 mgm. per gram of heart muscle per hour, the maximum being 4.11 mgm. and the minimum 2.06 mgm. No alteration could be detected in the rate of the beat or the rate of perfusion as a result of insulin. The results show clearly that insulin accelerates the rate at which glucose is removed from Locke's solution by the heart muscle and there can be little doubt that the same effects would be produced in other active tissues.

Attempts were made to demonstrate a similar disappearance of sugar in the case of the skeletal muscles, by perfusing the blood vessels of one hind leg of the cat or rabbit through the iliac artery with oxygenated Locke's solution or with defibrinated blood. Although every precaution was taken to make the perfusion as nearly physiological as possible with regard to temperature, pressure, oxygen supply and CO_2 tension, and to start it before the normal circulation through the limb had ceased, it was found impossible to prevent either considerable

edema of the muscles or the development of marked resistance to the perfusion. This made it useless to determine the rate of disappearance of glucose from the perfusion fluid.

These failures led us to see whether increased disappearance of glucose in the skeletal muscles might be demonstrated by comparing the percentage of sugar in samples of blood removed as nearly as possible simultaneously from the main artery and vein of the leg in anesthetized animals. At the same time blood was also collected from the portal vein. Comparison of the percentages of glucose in the three bloods, both under normal conditions and in conditions of hyperglycemia due to sugar injections, had previously been made by Macleod and Fulk (1917) and in arterial and venous blood by Henriques and Ege (1921). It was hoped that although the volume-flow of blood is so large that considerable alterations in the rate at which sugar leaves the blood might occur without causing any measurable change in the difference between the percentages in arterial and venous blood, insulin would accelerate the rate of disappearance sufficiently to reveal its hypoglycemic action. Such was found, however, not to be the case, either when the blood sugar to start with was about the normal level or when it was much above it as a result of injection of sugar, or of epinephrin.[5]

Although for various reasons, it would be of considerable interest to be able to demonstrate that insulin affects the rate of disappearance of glucose from fluid perfused through skeletal muscle, as can be done in the case of fluids perfused through the heart, the demonstration is not essential as proof that it has this effect.

The question narrows itself down to the cause for the lowering of the tension of glucose in the tissues. We have seen that glycogen is formed in the liver in depancreatized animals, when insulin is given along with carbohydrates, and that the respiratory quotient becomes changed in a manner to indicate that increased combustion of carbohydrates is occurring. It would be natural to suppose that the same effects must be produced in normal animals and so account for the disappearance of the glucose. We will now proceed to consider the observations which have been made to test this possibility.

1. The behavior of glycogen in normal animals following insulin. The observations have been made either by giving insulin along with carbohydrates to animals rendered free of glycogen by starvation coupled

[5] Even in depancreatized dogs insulin cannot be shown to cause a change in the difference between the sugar percentages in arterial and venous blood (McCormick).

with injections of epinephrin (McCormick, Macleod and O'Brien, 1923) or by giving it to glycogen-rich animals (Dudley and Marrian, 1923). In both groups of experiments glycogen was determined in the liver, muscles and heart at varying periods after giving the insulin and the results compared with those obtained on other animals that had been treated precisely in the same manner, except that insulin was not given. The results are shown in table 5.

TABLE 5

Effect of insulin on glycogen percentage in livers of previously starved rabbits

INSULIN	NO INSULIN	MANNER OF GIVING CARBOHYDRATE
1.48 C	4.16	Glucose subcutaneously at regular intervals during 4 hours
3.88	3.44	
2.72	4.76	
2.60 C	5.12	
2.90		
Average 2.72	4.37	
5.96	5.56	Glucose subcutaneously during 6 hours
3.08	8.70	
3.56		
Average 4.20	7.13	
2.00	3.00	Fed small ration oats for several days
2.30 C	3.65	
0.27	0.73	
0.03 C	1.48	
1.60	2.18	
0.80 C	1.18	
2.25 C	0.43	
Average 1.32	1.80	

In the observations of the last group the animals were first of all starved and then fed during several days with oats, each animal receiving the same quantity, which was about one-half of what the animal would voluntarily have taken. Insulin was also injected at intervals into half the number of rabbits in each experiment, the endeavor being made to give doses just insufficient to cause convulsions. When these did occur (indicated by *C* in the table) the animal was allowed to recover spontaneously. The figures on the same horizontal lines refer to observations made simultaneously. It is clear that less glycogen was

deposited in the livers of the animals receiving insulin. The results for muscles and heart were less decisive and do not permit of any definite conclusions.

2. The influence of insulin on the glycogen already present in the liver and muscles. Two rabbits were fed on carrots for a couple of days so as to enrich the glycogen stores. One of these was then injected with insulin until convulsions supervened when it was killed. The other was not given insulin but was kept without food during the time the first one was receiving it and was killed at the same time. The following illustrates the amounts of glycogen found (Dudley and Marrian):

	NORMAL RABBIT	INSULIN RABBIT
Liver	5.53	1.86
Heart	0.26	0.54
Skeletal muscle	0.57	0.0

There can be no doubt that insulin causes a rapid reduction in the amount of glycogen in the liver and may cause it almost, if not entirely, to disappear from the muscles. It seems to be the case, also, as pointed out by Dudley and Marrian (1923) that when convulsions supervene in well-fed animals no more than traces of glycogen may be present in the muscles, considerable amounts, however, still remaining in the liver.

Instead of glycogen being formed when insulin is given it is clear that the opposite occurs, and the reason for this disappearance is not far to seek, namely, conversion of glycogen into glucose to make good the loss of this sugar from the blood. We must conclude that at some stage after the blood sugar becomes reduced by insulin the glycogenolytic process in the liver is stimulated, probably through action. The nervous stimuli responsible for this effect are set up by lowering of the tension of glucose below a certain level in the nerve cells, and the efferent stimuli are carried to the liver through its thoracico-lumbar autonomic (sympathetic) nerve supply. Evidence for this is afforded by the fact, observed by Burn (1923), that the blood sugar following insulin falls much lower and remains depressed for a much longer period when the efferent nerves are paralyzed by ergotamine, which like ergotoxine, specifically acts on the nerve terminations of the thoracico lumbar autonomic nerves. It is possible also that the glycogenolytic impulses to the liver are reinforced by the influence of the lowered blood sugar acting directly on the liver cells. In any case there is no doubt that the glycogenolytic

process is stimulated and it remains to ascertain whether the process sets in at an early stage in the development of hypoglycemia, or only after a certain low threshold value of blood sugar has been reached. That the blood sugar, following insulin, immediately falls in glycogen-rich animals may depend either on the failure of afferent stimuli to be set up until this threshold is reached, or on the inadequacy of the supply of sugar from the liver to keep pace with the loss of sugar from the blood, even when glycogenolysis is stimulated to its maximum.

Further evidence that glycogen formation is not stimulated by insulin has been furnished by experiments with E. C. Noble (1923) in which the liver of the turtle was used. This consists of two lobes which are joined together only by a narrow strip of liver tissue, the blood supply of each lobe being carried mainly by separate vessels, the umbilical veins, into which cannulae can readily be inserted. The left lobe also receives some of its blood through numerous small veins running from the stomach, and the right lobe, through a vessel called the spermatic vein, which enters at its under surface. These anatomical conditions seem to offer an opportunity to study glycogen formation by comparing the amount of this substance in the two lobes, the one being used as a control and the other perfused with a solution containing the substance whose influence on glycogen formation it is desired to study, for example, insulin. The control lobe may either be removed at the start and the other perfused with Ringer's solution containing glucose and insulin, or both lobes may be simultaneously perfused, (after tying a ligature around the strip of tissue which connects them) under exactly similar conditions except that insulin is added to the fluid on one side only (cf. Grube, Schöndorff and Grebe, Nishi). The results have failed to demonstrate that insulin causes glycogen to be deposited, or its rate of disappearance to be stayed. There is normally a decided difference in the percentage of glycogen in the two lobes, and this makes the interpretation of the results obtained by the above methods somewhat difficult, but when a sufficient number of observations is taken into consideration this error is eliminated, and no effect of insulin can be made out.

The problem has also been investigated by observing the percentage or the total amount of sugar in the fluid after it has passed through the liver, before and after the addition of insulin, such a method having previously been employed by Snyder, Martin and Levin (1922) to investigate the glycogenolytic action of epinephrin. Under the conditions of perfusion obtaining in these experiments the liver constantly yields

some sugar to the perfusion fluid, indicating that a certain degree of *post-mortem* glycogenolysis is occurring. The addition of insulin to the perfusion fluid was not found to have any effect on the process. Although the results of these experiments are entirely conclusive, in showing that insulin has no effect on glycogen formation in the turtle liver, they could not in themselves be considered as final proof that insulin does not influence this process in other, especially warm-blooded, animals. This objection to the turtle experiments is justified on account of the fact, first clearly pointed out by A. Krogh, that the hypoglycemic effects of insulin are only very slowly developed on cold-blooded animals, such as the frog. We have also found that insulin does not cause hypoglycemia within several hours in turtles. The results of these experiments indicate that it is unlikely that insulin could be shown to affect glycogen formation in yeast.

2. The behavior of the respiratory quotient and the oxygen consumption following insulin. The first observations of the effects of insulin on the respiratory exchange in normal animals were made along with Pember and Dixon, and they revealed a decided increase both in R. Q. and in O_2 consumption, especially in dogs. The rise in R. Q. was particularly evident in animals in which this was low to start with—as a result of starvation—and it occurred, as a rule, within the first 2 hours following the injection of insulin, frequently falling back to the original level at a later stage. In one or two observations, on an animal that was frequently used for this work, the R. Q. actually rose for some time above unity (to 1.2). The observations on rabbits usually also showed an increase in R. Q. with little change, or it might be a decline, in the oxygen consumption. These results were interpreted as indicating an increase in the relative amount of carbohydrate undergoing combustion, and, possibly, a reduction of carbohydrate into fat-like substances, thus accounting for the elevation of R. Q. to over unity (Macleod, 1923). A similar process, it will be recalled, is held to be responsible for the high quotients observed in hibernating animals when they are ingesting large quantities of carbohydrates in the autumn. Later observations on mice by Dudley, Laidlaw, Trevan and Boock (1923) did not confirm the results of Pember and Dixon, both the consumption of oxygen and the production of carbon dioxide being found to become greatly depressed as a result of insulin. These workers found R. Q. sometimes to rise at first, this being due to a more rapid decrease in O_2 than in CO_2. Kellaway and Hughes (1923) found in a normal (starving) person that R. Q. became raised without a sufficient increase in O_2 consump-

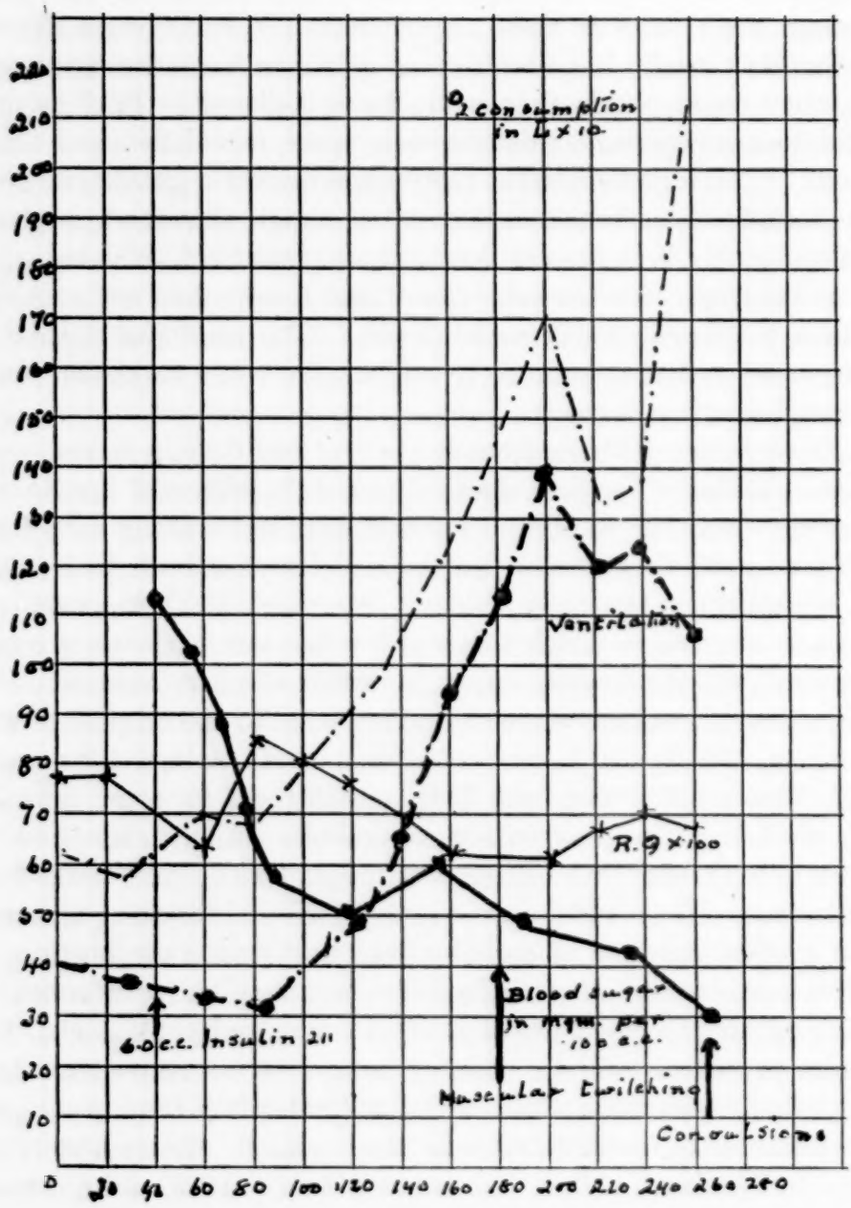
O2 consumption in L x 10
Ventilation
R.Q x 100
6.0 c.c. Insulin
Blood sugar in mgm. per 100 c.c.
Muscular twitching
Convulsions
0 30 40 60 80 100 120 140 160 180 200 220 240 260 280

Fig. 6. Curves showing effect on normal dog of injection of insulin. Heavy unbroken line—percentage of blood sugar. Thin unbroken line—respiratory quotient multiplied by 100. Heavy dot and dash line—Volume of expired air in litres per minute divided by 10. Thin dot and dash line—oxygen consumption in litres multiplied by 10.

Abscissa minutes from start of observation.

tion to account for it by increased conbustion of carbohydrate. These conflicting results prompted us, along with G. S. Eadie (1923), to repeat the observation on dogs, particular care being taken to find the relationship of the respiratory changes to the blood sugar, the reserve alkali of the blood, the rectal temperature and the rates of breathing and the pulse.

The results of a typical experiment on a *dog* are shown in the curves of figure 6. By the time the initial fall in blood sugar was completed there is seen to have been a perceptible increase in R. Q. but no change in any of the other values. As the hypoglycemia gradually became more profound the respiratory rate and volume and the oxygen consumption began to rise and they continued to do so until the observations had to be terminated on account of the incidence of convulsions. R. Q. did not remain elevated but returned to its normal level. It could be noted in this and in other similar observations (both on this and other animals) that the increase in O_2 consumption, respiratory volume, etc., became noticeable when the animal was becoming restless, indicating the onset of the premonitory symptoms of hypoglycemia, and that by the time the symptoms were marked, the values rose to several times their normal. The observations were repeated with the difference that whenever any signs of restlessness could be noted glucose was injected so as to remove them, with the result, shown in figure 7, that there still occurred a rise in R. Q. but practically no change in the other values. The rectal temperature was found to fall steadily after insulin with a temporary elevation following convulsive seizures.

Although restlessness and increased muscular activity are not so evident, as premonitory symptoms, of the convulsive seizures in *rabbits*, a decided increase in O_2 consumption was observed in an hour or so after injecting insulin, in about one-third of the observations. In about two-thirds of them the O_2 consumption either did not change or declined somewhat. The respiratory quotient, especially in previously starved animals, was usually found to rise. There is some difficulty in interpreting these results because of the confusion between effects which may be due to insulin and those consequent upon increased muscular activity. In order to eliminate the effect of muscular activity A. Krogh has recently observed the respiratory exchange following insulin in curarized rabbits, and has found that R. Q. becomes raised after about one-half hour, when the blood sugar has completed its initial fall, and that O_2 consumption remains unchanged, or declines only slightly. Both he and Dale (1923) interpret the results as showing that insulin shifts the metabolism

to one in which relatively more carbohydrate is being metabolized and Krogh points to the experiments of Ahlgren, already referred to (p. 46) as further evidence that insulin leads to conversion of glucose into less stable modifications, so that an increase occurs in its combustion. The quotient did not increase to such an extent as to indicate a reduction of carbohydrate to fat-like substances.

Because the increase in the respiratory quotient following insulin is not usually pronounced and is often transient, we must not lose sight

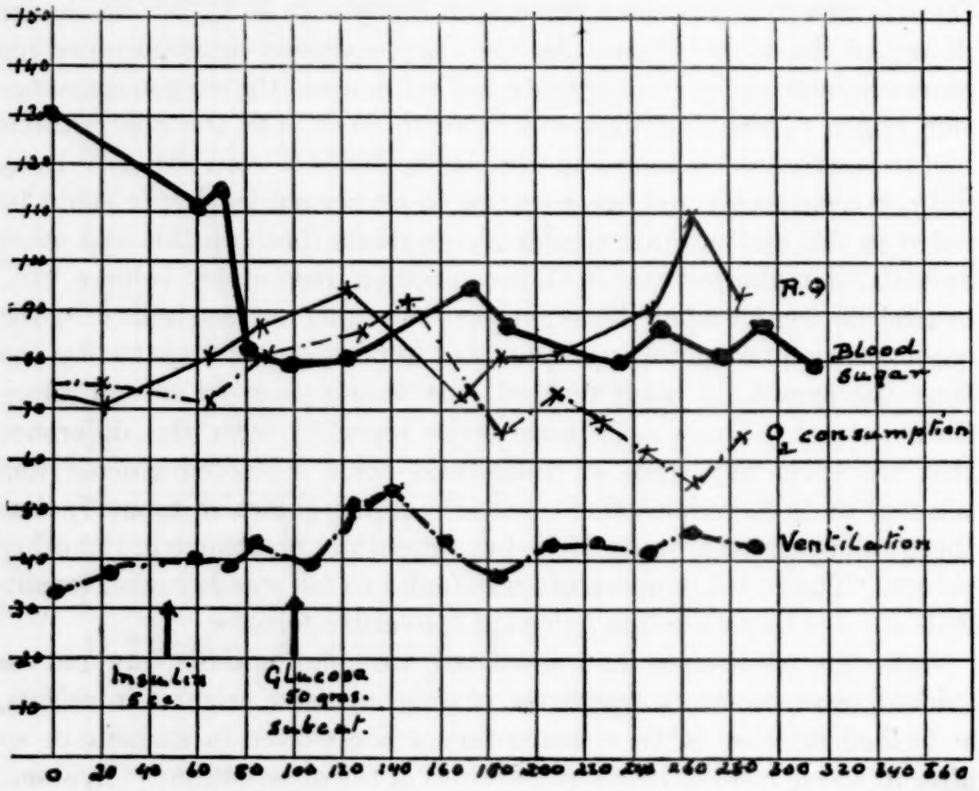

Fig. 7. Curves showing effect on normal dog of insulin followed by glucose. For intrepretation see legend of figure 6.

of the fact that it may be due to secondary conditions brought about by insulin, such as: *1*, the formation of acid substances which then expel CO_2 from the carbonates of the blood and tissues; *2*, primary excitement of the respiratory center causing a blowing off of CO_2; *3*, locking up of oxygen in the formation of some hitherto unidentified substance (see p. 38). With regard to the first possibility, no evidence could be obtained, of a change in the excretion of ammonia or in the titratable acidity of the urine or in the CO_2-combining power of the blood

in normal dogs given insulin; and with regard to the second it will be noted, by consulting figure 6, that although hyperpnea is an effect of insulin, R. Q. is actually falling at the time it develops. A certain amount of support for the third possibility is afforded by the observation that not infrequently we have noted that the arterial blood becomes visibly venous in color at the time hypoglycemic symptoms supervene.

Taking the available evidence as a whole it seems probable that insulin causes an alteration in the type of metabolism by bringing relatively more carbohydrate into the process. This does not necessarily mean that there must be an increase in O_2 consumption, indicating a greater production of energy, for in proportion as more carbohydrate is oxidized so may there be less oxidation of protein and fat. The change is qualitative rather than quantitative in nature: It is only when hyperexcitability of the neuro-muscular mechanism becomes a prominent symptom that such a marked increase in energy metabolism, as we have observed in dogs, can be demonstrated to occur. Sometimes, as in mice and to a less extent in rabbits, the energy expenditure may actually become depressed and as suggested by Dale this may occur when the supply of carbohydrate in the body is beginning to run low. As contributory evidence that relatively more carbohydrate is being consumed, following insulin, may be mentioned the fact, demonstrated by F. N. Allan, that a decrease occurs in the daily output of nitrogen when insulin is given to normal dogs in amounts sufficient to produce preconvulsive symptoms. This is illustrated in the accompanying table of results:

The daily excretion of nitrogen and of urea are seen to fall on the days insulin was given, particularly when the animal was on a high protein diet, without there being any change in that of creatinin. The excretion of ammonia and acid, as already pointed out, is not significantly affected.

Taking the foregoing results as a whole, the outstanding facts are that insulin causes the sugar of the blood (and tissues) to diminish both in the normal and diabetic animal, partly because of an increase in the relative amount of carbohydrate that is metabolized and partly because some of the glucose is converted into non-saccharine material, which is mainly glycogen in diabetes, but is some other substance in the normal animal. In the latter case insulin actually causes the glycogen stores to become depleted because of the demand for glucose which it sets up, partly to form this unknown substance and partly to be oxidized. We fail to identify the substance because it has lost the reducing properties upon which the identification of sugars depends and, at the

same time, is not precipitated by alcohol as glycogen after treatment of the tissue with caustic alkali. It will be remembered that Pember and Dixon, in the earlier observations on respiratory exchange, found that the respiratory quotient, following insulin, was sometimes raised to above unity and this can be explained only by supposing that some sub-

TABLE 6

DATE	N INTAKE (1)	N BALANCE (2)	EXCRETION						
			Total N grams	Urea		Ammonia		Creatinin grams	Titratable acidity (Folin)
				Grams	Per cent of total N	Grams	Per cent of total N		
Dog C. High protein diet. 500 grams meat									
Jan. 9						0.48	3.28	0.326	
Jan. 10	16.25	−0.54	15.79	14.52	92.06	0.58	3.68	0.375	133.0
Jan. 11	16.25	+0.85	16.10			0.53	3.29		138.6
Jan. 12	16.25	−0.46	15.71*	14.40	91.60	0.55	3.50	0.385	121.0
Jan. 13	16.25	+2.05	13.20	11.38	86.20	0.45	3.41	0.252	
Jan. 15	16.25	+0.29	14.96					0.326	
Jan. 16	16.25	+0.81	14.44	12.76	88.37	0.44	3.06	0.463	127.6
Jan. 31	16.25	−1.89	17.14			0.54	3.17	0.326	146.0
Feb. 1	16.25	−0.83	16.08			0.53	3.33	0.351	134.6
Feb. 2	16.25	−1.18	16.43	14.76	89.84	0.53	3.23	0.326	140.6
Feb. 3	16.25	+1.73	13.52*	11.67	86.52	0.58	4.32	0.329	
Feb. 4	16.25	+0.81	14.44	12.74	88.35	0.44	3.07	0.326	138.4
Feb. 5	16.25	−1.40	16.65	14.79	88.80	0.50	3.00	0.326	123.0
Feb. 7	16.25	−2.51	17.76	16.53	93.07	0.59	3.33	0.336	153.0
Feb. 8	16.25	−1.68	16.93			0.53	3.11	0.330	145.2
Feb. 9	16.25	−0.60	15.85*	13.65	86.14	0.52	3.25	0.315	126.0
Feb. 10	16.25	+0.69	14.56	12.99	89.21	0.49	3.38	0.315	135.6
Dog I. Mixed diet. 100 grams meat, 150 grams oatmeal									
Mar. 26	7.15	+1.64	4.51						
Mar. 27	7.15	+1.64	4.51	3.65	80.93	0.33	7.32		156.0
Mar. 28	7.15	+0.59	5.56*	4.49	80.60	0.43	7.72		198.0
Mar. 29	7.15	+2.17	3.98						174.0
Mar. 30	7.15	+1.75	4.40						

* Insulin given here.

stance containing less oxygen than carbohydrates is being formed out of them, and one naturally thinks of fatty acid as this substance. By chemical examination of the combined livers of several mice given insulin and of an equal number not so treated, Dudley and Marrian (1923)

were unable to detect any difference, either in the total amount or in the iodine value of the fatty acid, showing that if insulin causes reduction of carbohydrates to fats this process does not occur in the liver. But it is possible that the substance that is formed is not fatty acid as such, so that it would not be included in the determinations. Winter and Smith (1923) have found that running parallel with the diminution in the concentration of sugar that of the inorganic phosphates also becomes reduced in the blood, which would seem to indicate that the new substance is a compound containing a phosphoric acid group.

These difficulties in accounting for the sugar which disappears, as a result of insulin, recall the equally mysterious disappearance of carbohydrate observed by Bang, (1913) Meltzer and Kleiner, and Palmer to occur when sugar in varying quantities is injected into normal animals. Even when the entire body is examined for sugar and glycogen in a few minutes following the injection, it is impossible to account at the most for more than about 70 per cent of the injected carbohydrate.

THE SOURCE OF INSULIN

It is a tribute to the value of painstaking work in normal and pathological anatomy that within a few years of Mering and Minkowski's discovery, that pancreatectomy is followed by diabetes, the hypothesis that the Islets of Langerhans are the source of insulin should have become almost universally accepted. It would go beyond the scope of this article to recount the various investigations supporting this conclusion, a full review of those depending on pathological evidence being given by Opie, W. G. MacCallum, etc. Very strong support for the hypothesis is also afforded by experimental investigations in which it was shown that diabetes does not supervene after ligation of the ducts of the pancreas, or after transplanting a portion of it under the skin of the abdomen, so as to form a graft. In both these experiments the acinar cells, which produce the digestive juice of the gland, ultimately disappear whereas the islet cells, after a preliminary stage of degeneration, become regenerated and ultimately are the only epithelial tissue left in the residue of the gland. A full acccount of these changes is given by R. R. Bensley (1911) and by F. M. Allen (1913). Assuming that the pancreas owes its anti-diabetic properties to the fact that it produces insulin, there seemed to be little doubt that the islets must be its source. The experiments of Banting and Best give strong support to this view but they do not absolutely prove it because in the time which

was allowed to elapse after ligation of the ducts (5 weeks), there would probably still remain in the pancreas some regenerated acinar cells which might be the source of the insulin.

Another method of approaching the problem depends on the discovery made in 1899 by Diamare and confirmed by Laguesse (1906) that the islet tissue in certain of the Teleostei exists separate and apart from the acinar tissue, forming distinct and often large encapsulated glands situated in the mesentery. In 1906 Rennie described the exact position of these glands—the largest of which he called "principal islets"—in a large number of fishes, and he showed, by the aid of the histological methods then available, that the amount of acinar tissue associated with them is extremely small, although some is usually present. Slater Jackson (1923) has recently shown that when the methods for differential staining described by Lane, Bensley, Martin, etc., are used, the cells of the principal islets behave like those of the islets of Langerhans. Diamare and Kuliabko and later Rennie and Fraser (1907) realizing the significance of the anatomical findings in connection with the problem of the source of insulin, studied the effect of extracts of the principal islets on solutions of glucose hoping to find that they might accelerate glycolysis, but with negative results. Rennie and Fraser also investigated the possible value of the islets in the treatment of diabetes in man. They administered either the raw glands or freshly prepared extracts of them by mouth to diabetic patients and observed the effects on the glycosuria and the general symptoms of the disease, but, for reasons which are now perfectly understandable, they did not succeed in supplying convincing evidence that the diabetic condition was thereby relieved. In one patient they injected subcutaneously saline extracts of the islets but the toxic symptoms which followed compelled them to abandon further investigation in this direction.

In the light of Banting and Best's discovery of the presence of insulin in extracts of duct-ligated pancreas repetition of Rennie and Fraser's investigations seemed advisable. In the angler or devil fish (Lophius piscatorius), the sculpin (Myoxocephalus) and the flounder (Pleuronectidae) the islets are relatively large and the acinar tissue exists as narrow bands along the branches of the mesenteric veins. Extracts were prepared from islets and acinar tissue, separately, by extracting them with about 60 per cent acid alcohol, blowing off the alcohol, removing excess of fat by ether, then heating the watery solution and filtering. The resulting solutions, were then injected in rabbits with results like those in table 7.

Similar extracts after Berkfelding have been found of clinical value, and free from substances having a local or general toxic action. It is clear that the islets give large yields of insulin and that this is absent from the acinar tissue. Indeed, in some cases extracts of this tissue may actually cause the blood sugar to increase decidedly. Since the amount of acinar tissue associated with the principal islets is relatively very small and islet cells are almost, if not entirely, absent in the bands

TABLE 7

Effect of extracts of principal islets and of pancreatic tissue on blood sugar of normal rabbits

I. *Islets.* Weight of (alcohol soaked) islets, 0.15 gram. Total volume of extract, 12 cc.

August 23	10:45 a.m.—0.108 per cent blood sugar
	10:50 a.m.—Injection of 2 cc.
	12:00 noon—0.042 per cent blood sugar
	12:40 p.m.—Rabbit hyperexcitable and on removal from cage developed convulsions
	12:45 p.m.—0.024 per cent blood sugar
	12:47 p.m.—Injected 2 grams dextrose
	1:20 p.m.—0.054 per cent blood sugar. Rabbit apparently normal
	3:20 p.m.—Rabbit again in convulsion
	3:25 p.m.—0.025 per cent blood sugar
	3:30 p.m.—Injected 2 grams dextrose. After this and during the evening the animal was normal though drowsy
Next morning—Rabbit normal	

II. *Pancreatic tissue.* (All visible pancreatic tissue of 2–35 pound fish)

August 23	11:05 a.m.—0.124 per cent blood sugar
	11:15 a.m.—Injection of entire extract
	12:15 p.m.—0.124 per cent blood sugar
	1:15 p.m.—0.110 per cent blood sugar
	3:40 p.m.—0.112 per cent blood sugar
At no time did the animal show any symptoms	

of acinar tissue, these results furnish incontrovertible evidence that insulin, as indeed its name implies, is derived exclusively from the islets. It is probably safe to assume that it is the β cells that are responsible, for not only are these decidedly much more numerous than the α cells, at least in mammals, but their granules are readily soluble in dilute alcohol in which the granules of the α cells are insoluble (cf. Lane). They are said also to be the cells which primarily become the seat of degeneration in diabetes (cf. F. M. Allan).

Quite apart from the theoretical interest of the results they have a practical value in that they show that fish may be an important source of insulin for clinical purposes. N. A. McCormick and E. C. Noble have investigated this possibility at the Atlantic laboratory of the Biological Board of Canada, and have found that the principal islets are quite readily identified and can be removed practically free of acinar tissue, particularly in the cod (Gadus callarias) and the halibut (Hipoglossus). In the cod the principal islet is situated at the top of the gall bladder and is shaped like a "cocked hat." It is of a pink color on account of its rich blood supply and can very readily be identified and removed. These workers have succeeded in preparing from large quantities of islets, removed by fishermen, by a simple and inexpensive method of extraction, highly potent insulin which has been found to be suitable for clinical use on diabetic patients. To illustrate the yield of insulin from these sources the following table may be of interest.

NUMBER OF FISH USED	TOTAL WEIGHT OF FISH (APPROX.)	WEIGHT OF ISLETS AFTER ALCOHOL	TOTAL VOLUME OF EXTRACT	COMPUTED UNITS OF INSULIN PER GRAM OF ISLETS
	pounds	*grams*	*cc.*	
20 large	325	4.4	5.5	22
300 small	2,000	3.8	6.6	14
2400 medium	27,000	66.7	132	20
		164		15

Considering that the islets were removed from the fish by unskilled labor after the fish had been brought to shore, and were kept in faintly acid alcohol for several days before the extracts were prepared, these yields of insulin are truly remarkable.

In certain others of the Teleostei, the islets and the acini are not so decidedly separated. In the lake trout (Cristivomer Namaycush), for example, both islets and acini are abundant in the fat which lies between the very numerous pyloric caeca. In sections across this region clumps of acinar tissue and small islets more or less free of acinar cells are seen embedded in the fat (D. J. Bowie). Extracts of this region, prepared by acid alcohol, are strong in insulin. In the carp (Cyprimus carpio) on the other hand, in which there are no pyloric caeca, the

pancreas is largely bound up with the liver, both acini and islets being readily distinguished in close relation which the liver tissue. An extract of this hepatopancreas contains large quantities of insulin.[6]

In the Elasmobranchi (skate and dogfish) the relationship of the acinar to the islet tissue is quite different from those in the Teleostei, being very similar to that existing in mammals. There is a well-defined pancreas attached to the first portion of the small intestine, and, on section, it is found to consist of typical acinar tissue with the islet tissue closely associated with and often continuous with the epithelium of the main ducts. This islet tissue has been shown by Slater Jackson (1922) to contain the typical α and β cells of the mammalian islets. This evident attachment of the islet cells to the ducts is of especial interest since a similar close relationship is evident in the regenerated islet tissue which is seen in the residue of pancreas remaining several months after ligation of the ducts in mammals (R. R. Bensley, 1914). An extract of the pancreas of the skate and, for some unexplained reason, to a less extent in the dog fish (Squalus) contains large amounts of insulin but it is not probable, because of the association of acinar tissue, that the pancreas of these fishes, will prove to be of any more value as raw material for the manufacture of insulin than the pancreas of slaughter-house animals. The chief interest of these observations, apart from their possible practical value, rests on the evidence which they afford that insulin is, exclusively, a secretion of the islet tissue.

REMARKS ON THE CHEMISTRY OF INSULIN AND INSULIN-LIKE SUBSTANCES. No attempt will be made here to review in detail the chemical reactions and properties of insulin, or the methods used for its preparation. Work in these fields has been done, particularly by Collip (1922), Macleod and Best (1922), Doisy, Somogyi and Schaffer (1923), Malony and Findlay (1923), H. W. Dudley (1923), Widmark (1923), Winter and Smith (1923), and reference to their papers will be found in the bibliography to this article. Briefly stated, it has been found when the original insulin, prepared by fractional precipitation with alcohol according to Collip's directions, is further purified, as by Dudley's process—in which a picrate and finally a hydrochloride is formed—or by Doisy, Somogyi and Shaffer's method—in which it is precipitated at the iso-electric point —that the following reactions are obtained.[7] The solution can be evap-

[6] A unit of insulin is one-third the amount which is capable of lowering the blood sugar to the convulsive level (0.045 per cent) within 5 hours after injection.

[7] This account is mainly taken from the author's lecture before the Eleventh International Congress of Physiology at Edinburgh July, 1923.

orated to dryness *in vacuo* without loss of potency, and the powder contains about 14 per cent of nitrogen, is free from phosphorus (Doisy, Somogyi and Shaffer), but contains sulphur. A solution gives a distinct biuret test, but only faintly, those of Millon and Hopkins-Cole. It gives the Pauly reaction (iminazole ring). The insulin is readily absorbed by kaolin and charcoal, and considerable losses are apt to occur in passing it through a porcelain filter. It does not dialyze through membranes or collodion sacs; it is precipitated by half-saturation with ammonium sulphate, by barium salts of weak acids, by picric acid and by uranium acetate. The solubility in water and alcohol is determined by the H-ion concentration, the iso-electric point being at a pH below 7. The insulin is relatively stable to mild oxidizing and reducing agents. Insulin is also soluble in phenol and the cresols, glacial acetic acid, and formamide. It is insoluble in most other organic solvents (Malony, 1923, Widmark, 1923).

These reactions, and its destruction by pepsin and trypsin, suggest that insulin "is probably a substance having a very complex structure and protein like in its nature" (Dudley), but it is possible that it is really only adherent to this protein, and is itself of much simpler chemical structure. This view is supported by the fact that we have been unable on several occasions to detect any biuret reaction with highly potent insulin prepared from the pancreas of the skate or to obtain from this source precipitates containing it by means of high concentrations of alcohol, or by adjustment of the iso-electric point. Whether it is really a protein or not, it is significant that all the methods now used for its purification are based on reactions that are characteristic of colloids. Thus, precipitation at the iso-electric point (Doisy, Somogyi and Shaffer, 1922), or by picric acid followed by the regeneration from the picrate as a hydrochloride (Dudley, 1923), or by benzoic acid (Malony), or by half saturation with ammonium sulphate.

It may be assumed that insulin is present in all animals, and since structures similar to the Islets of Langerhans have not been described in the invertebrates it is likely that in them its site of production is delegated to other structures. What these may be in the more highly developed invertebrates is as yet undetermined, but it is of interest that Collip (1923), shortly after isolating insulin from mammalian pancreas, prepared it from clams, and suggested that it must be present wherever glycogen is found. Later he prepared a substance like it from yeast, thus confirming Winter and Smith (1923). The insulin prepared from these sources appears to differ from that prepared from pancreas, in

that its action in lowering the blood sugar is delayed, sometimes for a day or so, which is also the case with an insulin-like substance prepared by Collip from various vegetable sources and styled by him, in recognition of these differences, "Glucokinin." Best and Scott (1923) have also succeeded in preparing insulin, or insulin-like substances, from other sources including various organs and tissues both of normal and diabetic animals. It would appear, therefore, that the distribution of hormones capable of accelerating the disappearance of glucose from the blood is widespread, not only in the animal kingdom but in the vegetable as well, but it is probable that these do not all have the same physiological action. In the investigation of this problem we must bear in mind that many substances have been known for long to be capable of lowering the blood sugar; for example, peptone, hydrazin, certain mineral salts, phloridzin, etc. All of these also cause glycogen to disappear from the liver, and indeed certain of them cause structural changes in this organ. Alkalies also cause some reduction (Murlin, Underhill, Macleod and Fulk). Although it would certainly be desirable to have substances like glucokinin having a more prolonged action than insulin in depressing the blood sugar, we must exercise some caution in recommending them for clinical use until it is certain that they have no deleterious effects on the liver. It is possible also that these substances merely stimulate the secretion of insulin from the pancreas of the animal into which they are injected, or serve as precursors for its production. If such be the case, then they can have little value in diabetes. It must be remembered also that extreme lowering of blood pressure is associated with hypoglycemia.[8]

Collip has more recently shown that when the blood of a rabbit in which the sugar has been reduced by injection of glucokinin, or of other hypoglycemia-producing agents, is injected into a normal rabbit the blood sugar becomes rapidly reduced. If the blood of this rabbit be injected into a third animal, the same result is obtained, and also in a fourth, a fifth, and so on. The significance of these results is unknown but it is interesting that they are obtained with guanidine sulphate as well as with glucokinin. When hypoglycemic symptoms develop in animals treated in this way glucose only temporarily removes the symptoms.

Finally it may be stated that typical insulin should exhibit the following physiological properties:

[8] The delayed action may sometimes be due to the presence of hyperglycemia-producing extractives, such as occur in the pancreas proper and the liver of certain Teleostei (Macleod, McCormick and Noble).

1. When given to normal animals (rabbits), it should cause immediate reduction of the blood sugar and when hypoglycemic symptoms supervene these should be immediately and permanently removable by injections of glucose.

2. When given to diabetic (depancreatized) animals the symptoms (hyperglycemia, glycosuria, acetonuria) should immediately disappear and the general condition greatly improve; and when carbohydrate is also given R. Q. should rise decidedly and glycogen be deposited in the liver.

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BASAL METABOLISM

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The literature on what was originally classified under the term respiratory metabolism and more recently as basal metabolism has fortunately been reviewed extensively at short intervals, so that many authoritative résumés are available. As these reviews are listed at the beginning of our bibliography we only need refer here to the monograph of Grafe which has appeared during the last year in Ergebnisse der Physiologie. This monograph is excellent and comprehensive, and includes both European and American literature to date.

In order to avoid unnecessary reduplication we have not, therefore, attempted to cover all the literature. Instead we are presenting the status of the subject as it now is in this country, and have limited our discussion to those problems that are under active investigation.

We have departed from the usual arrangement of the bibliography in that we have, through the kindness of the directors of the various laboratories, obtained and placed together the publications of each. In the case of the work from the laboratories of Benedict and Lusk we have included all their articles dealing in any way with metabolism, as we believed that it would be of great help and interest to other investigators to have available a complete list of their publications.

Following the bibliographies from the various metabolism laboratories we have placed the more important of the very large number of papers on metabolism which are essentially clinical in nature. Although considerable pains have been taken to include those which have a physiologic bearing, we fear that some may have been omitted by mistake, for which we apologize.

APPARATUS. The science of metabolism originated with the investigations of Lavoisier in 1777. Although the details of the forms of respiration apparatus used by him and his collaborators, Laplace and Seguin, are unknown yet it seems probable, as pointed out by Tigerstedt, that they made use of the two principles which have formed the basis on which have been developed all subsequent types of apparatus for the determination of the respiratory exchange.

1. The closed circuit type of apparatus. In this method the subject is placed in or connected with a circulating stream of air in a closed system from which the carbon dioxide eliminated is absorbed for determination as formed and oxygen is added quantitatively to replace that consumed and to maintain the volume practically constant and is usually referred to as the Regnault-Reiset principle.

2. The open circuit type of apparatus. In this method the subject is placed in or connected with a measured stream of pure air and an aliquot part of out-going or expired air is then analyzed to determine the amount of carbon dioxid given off and oxygen absorbed therefrom by the organism and was first extensively used by Pettenkofer and Voit.

In both types of apparatus the first problem that arose was the connection between the subject and the apparatus. This was solved either by placing the subject entirely within the apparatus or by connecting the apparatus directly to the respiratory tract by mask, mouth, nose piece or tracheal cannula. Both these methods were used by Lavoisier. To-day the choice between them depends primarily on whether the contemplated experiments are to be of long or short duration.

By placing the subject inside the apparatus experiments can be performed with ease and comfort for many hours on man, and for many days on animals. The advantages of experiments of long duration lie primarily in the fact that, in addition to a determination of the respiratory exchange, an elemental analysis of the food, urine and feces can be made and a balance between the carbon, hydrogen, nitrogen and oxygen intake and output determined over one or more days, both under fasting conditions and after the ingestion of various types of food. Finally, in addition to the above, calorimetric appliances can be installed for measuring directly the quantity of heat eliminated, whereby it has been possible to prove that the law of the conservation of energy is valid in man and animals, in health and in disease.

By making a connection directly with the air passage either by mouth piece or other appliances, experiments of short duration only can be carried out. Until it was proved that abnormalities in the character of the respiration caused by a mouth piece or mask would not seriously alter the results, this method was strenuously objected to on theoretic grounds, which were in part justified in the earlier forms of these attachments because the tubing, connections and valves were not large enough to eliminate errors caused by resistance to inspiration and expiration. Furthermore, until the fundamental laws of metabolism

were established by experiments of long duration, the short experiments of ten to twenty minutes' duration were of little value. To-day, however, the short period experiment is of great value, first in completing our knowledge of the details of many fundamental proble s, and second in its clinical application in scientific medicine.

In general, in the closed circuit systems, the amount of carbon dioxid has been determined by passing the air through some form of absorber, the increase in weight of which gives the quantity eliminated; in the larger forms of apparatus the carbon dioxid content of the air in the experimental chamber had also to be determined at the beginning and end, and allowance made for changes in the percentage concentration. The accurate determination of the carbon dioxid elimination has been comparatively easy with all forms of apparatus, and in consequence this factor only was often determined. On account of the storage capacity of the body for carbon dioxid, short period experiments in which only this factor is determined are very unreliable, because a slight alteration in the depth and rate of respiration may materially affect the apparent production of carbon dioxid. In experiments of more than an hour's duration the temporary variation between production and elimination is too small to produce any appreciable effect on the total quantity of carbon dioxid. However, the information obtained from merely the carbon dioxid determination even if technically accurate, is of limited value because the calorific value of a liter of carbon dioxid varies as much as 31 per cent, depending on the character of food burned.

The calorific value of a liter of carbon dioxid when fat (R.Q. 0.71) is burned is 6.61 calories and when carbohydrate (R.Q. 1.00) is burned is 5.05 calories, or a difference of 1.56 calories, or with carbohydrate as the standard, the increase in the calorific value of carbon dioxid is 31 per cent. When, however, oxygen is used, the changes due to the character of the food burned are much less; the calorific value of a liter of oxygen when fat (R.Q. 0.71) is burned is 4.69 calories and when carbohydrate (R.Q. 1.00) is burned it is 5.05 calories, or a difference of 0.36 calories, or with carbohydrate as standard, the decrease in the calorific value of oxygen is only 7 per cent. It is obvious, therefore, that the carbon dioxid determination alone is unsatisfactory.

The usual method of determining the oxygen consumption in the closed system has been to add a measured volume of pure oxygen to keep the apparent volume of the system constant. The quantity of oxygen added was determined either by passing it through a meter

or by determining the loss in weight of the compressed oxygen cylinder. There was never any difficulty about determining with accuracy the amount of oxygen added, or in keeping the apparent volume of the apparatus constant. Great difficulty arose, however, from variations in temperature, pressure, and vapor tension, causing large fluctuations in the true volume of air in the apparatus. The larger the apparatus the greater the variations and the greater the difficulty in determining with accuracy the average temperature, pressure and moisture in the chamber. Slight variations from the true average amounting to a tenth of a degree in temperature, or of a millimeter in pressure, cause marked errors in the oxygen determination. Only since the closed circuit system has been reduced to a very small volume, a few liters, for a subject as large as a man, has this serious difficulty been entirely overcome except in those forms of respiration apparatus which are also direct calorimeters, and in which the temperature can be absolutely controlled. Likewise in the larger forms of apparatus it is often necessary, by very exact gas-analysis, to determine the oxygen as well as the carbon dioxid content of the apparatus at the beginning and end of the experimental period, and make appropriate corrections.

The general methods, therefore, in closed circuit types of apparatus are the gravimetric determination of carbon dioxid, and the volumetric determination of the oxygen. Under these circumstances the influence of temperature, vapor tension and pressure within the apparatus will not affect the gravimetric carbon dioxid value, but will fall entirely on the volumetric oxygen determination. If the volume of the apparatus is large, these factors may produce great variations in the actual volume of air in the chamber and will affect proportionately the oxygen determination, and render the latter and the respiratory quotient untrustworthy.

In the large open circuit type in which the subject is enclosed a constant stream of pure outdoor air is passed through the apparatus. For experiments of long duration it is impossible to collect all the expired air, therefore the total amount pumped through the apparatus is measured and an aliquot sample of the outgoing air is obtained by a miniature system of pumps and meters, which work in unison with the large pump and large gas meter of the ventilating system.

For short period experiments all the expired air is collected in a gasometer by means of a mask or mouth piece with valves, the air is measured, and a sample taken for analysis of its carbon dioxid and oxygen content in an accurate volumetric gas-analysis apparatus. If the

volumetric analyses are carefully made, the resulting respiratory quotients are accurate, because variations in the temperature, vapor tension and pressure affect alike the carbon dioxid and oxygen determination, and thus do not influence the ratio between the two, as is the case when one is determined gravimetrically and the other volumetrically.

Tigerstedt has given a very interesting historical account of the development up to 1911 of the various types of respiration apparatus, with a quite detailed description of the various modifications and with complete reference to the literature. Only these forms of apparatus that are in common use today will be mentioned here in detail.

The best modern type of closed circuit apparatus developed on the Regnault-Reiset principle is the universal respiration apparatus of Benedict. By means of a rotary blower driven by an electric motor a stream of air passes in a continuous circuit through the apparatus; the carbon dioxid is absorbed from the air by soda lime, the air first being dried by passing through sulphuric acid, and any water lost by the soda lime is recovered by a second absorber containing sulphuric acid; the gain in weight of the soda lime and second sulphuric acid container gives the carbon dioxid elimination. A very delicate spirometer measures changes in volume of the apparatus, and the amount of oxygen added to the system is determined by an accurate gas meter. The apparatus can be used with a mouth piece, or it can be connected with a chamber as described by Benedict and Tompkins; when, however, a chamber or box is used very careful temperature and pressure corrections must be made to obtain the true volume of air, slight errors in which produce a marked effect on the apparent volume of oxygen used. The modification, as described by Benedict and Talbot, is a very satisfactory method for babies and infants. The Benedict universal table is an admirable and excellent piece of apparatus. It is, however, expensive, especially when combined with the cot chamber, baby or animal box. Unfortunately in this type of apparatus, the major errors fall on the oxygen value, and therefore the most expert manipulation and care are necessary in order to obtain reliable respiratory quotients.

In the last few years several "simplified" models have been introduced by Benedict in an attempt to produce an inexpensive and easily manipulated closed circuit apparatus of sufficient accuracy for clinical use; the most satisfactory of these models is the Benedict-Roth, in which the complicating feature of the motor is avoided. In these

clinical forms no attempt is made to determine the carbon dioxid, and the estimation of the metabolic rate is based on the oxygen consumption determined by the decrease in volume of the apparatus, as shown by the fall of the spirometer, and calculating the heat production on the basis of a constant respiratory quotient assumed as 0.82. If the apparatus is used with a recording drum, a very satisfactory determination of the basal metabolic rate can be obtained.

A similar and inexpensive type of apparatus for clinical work has been developed by Krogh. Its advantages over the Benedict-Roth lie largely in the fact that the chamber for soda lime is larger and more readily changed. Furthermore the recording drum is an integral part of the apparatus, and no determination can be made except by obtaining a graphic record of the respiration and decrease in volume of the apparatus from the absorption of oxygen. Neither the Benedict portable, the Benedict-Roth, or the Krogh apparatus should ever be used without obtaining a graphic record of the respirations; the fact that in the Krogh apparatus no other means is provided of making readings except the graphic record, is a distinct practical advantage in an apparatus intended for indiscriminate use.

The micro-respiration apparatus devised by Krogh is intended for very exact studies on the respiratory exchange of small animals and is suitable for very exact scientific investigation of the character of the metabolism.

The cot-chamber of Benedict and Tompkins and of Benedict and Talbot commonly used as a closed circuit apparatus can be arranged for the open circuit method by attaching the devices for obtaining an aliquot sample of the outgoing air according to the methods used by Pettenkofer, Voit, Jacquet or Krogh and Lindhard. The latter have described, recently, a very exact technic by which the chamber open circuit method can be used for work experiments. The utmost exactness in gas-analysis must be used in these larger forms of apparatus, on account of the slight changes in the concentration of carbon dioxid and oxygen, and for this purpose Krogh devised a very exact but exceedingly complicated form of gas-analysis apparatus. The method, has, however, in Krogh's hands been most satisfactory.

The most commonly used open circuit apparatus is the gasometer in which is collected by means of mouth piece or mask and suitable valves, the total volume of expired air, a sample of which is analyzed in a Haldane gas-analysis apparatus. This method is particularly suited for the scientific study of the respiratory metabolism in man and

in dogs, and is also the best form of clinical apparatus, if it is desired to obtain the respiratory quotient as well as the basal metabolic rate. The gasometer method was first used by Speck (1871) although in this country the credit has generally been given to Tissot, who, however, made many important improvements in the gasometer and valves, and added electric attachments for recording the respiratory movements, concerning which he was much more interested than in determining the heat production. Carpenter suggested the use of the Siebe-Gorman half-mask. Further improvements were made by Boothby and Sandiford in the construction of the gasometer and the arrangement of the inspiratory, expiratory and control valves, and they have described in detail the technic of basal metabolic rate determinations by this method. Their recent changes in technic, necessary in order to render the method suitable for dogs, has been described by Boothby and Sandiford, and by Kitchen. The gasometer method can be used for repeated tests at short intervals, thus allowing the accurate determination of many points of the metabolism curve following the ingestion of food, drugs or the effect of work. Its disadvantage lies in the fact that accurate gas-analysis on the Haldane gas-analysis apparatus is required. This, however, while requiring time and patience to learn, is such that it can be readily acquired by a technician, if she is properly instructed.

For experiments outside the laboratory a very convenient form of the open circuit type of apparatus is that described by Douglas: the expired air is collected by means of especially designed valves and connections in a large rubber bag suitable for carrying on the back, and of a much more convenient shape than that used by Regnard. The expired air, after collection, is passed through a dry gas meter to determine the volume, and a sample taken for analysis on the Haldane gas-analysis apparatus The mouth piece, valves and connections, as well as the light weight of the bag, render the apparatus particularly valuable for outdoor work experiments. In this respect it is more convenient than the portable form of the Zuntz-Geppert apparatus, which consists of a dry gas-meter strapped to the back and the continuous collection of an aliquot part of the expired air made by an appropriate connection of the sampling device to the axis of the gas meter, according to the scheme originally used by Pettenkofer.

The open circuit apparatus, described by Haldane for use with small animals, is simple and quite accurate for the total heat production. However, as the oxygen is indirectly determined by subtracting the

loss in weight of the animal and container from the gain in weight of the carbon dioxid and water vapor absorbers, the respiratory quotient may be considerably disturbed by the fact that a large error may result from the indirect method of oxygen determination, on account of the difficulty of accurately measuring the water vapor. If, however, its use is limited, as originally designed, to small animals which can be weighed with the animal chamber on delicate balances, the method is quite satisfactory. Marine has recently described a slightly modified form of this apparatus.

STANDARD CONDITIONS FOR DETERMINATION OF BASAL METABOLIC RATE. In the discussion of the various factors affecting the metabolic rate it is assumed that technical errors are excluded by careful and accurate work, and that the determinations are made under the standard conditions unless the problem being investigated is a determination of the influence on the metabolism of one of these conditions. The accepted standard conditions for the determination of the basal metabolic rate and the reasons therefore, are briefly as follows:

1. Post-absorptive condition. It is essential that the patient shall have fasted from twelve to eighteen hours after the last meal. Soderstrom, Barr and Du Bois and C. G. and F. G. Benedict have studied the effect of a small practically non-stimulating standard breakfast, consisting in the case of the former, of a slice of bread with 8 grams butter, one cup of caffein-free coffee with 10 grams cane sugar and 66 cc. milk, and in the latter of one cup of caffein-free coffee, saccharin, 30 grams of medium cream and 25 grams potato chips. With neither type of breakfast was any noteworthy effect demonstrable in the heat production after the second hour. In our own laboratory, however, we have not considered it wise to permit any food, as experience has shown that in the case of patients it is impossible to control the quality and the amount taken, if any food at all is allowed. We have been forced to adopt the practice of sending the patient away to return the next morning without breakfast, if any food has been taken. Once the bars are let down greater and greater carelessness develops, and materially inaccurate results are obtained in an increasing proportion of tests.

2. Muscular and mental repose. Complete absence of muscular movement is admittedly essential to accurate determinations, and is only rarely difficult to obtain even in children. In certain subjects it is quite difficult to obtain muscular relaxation without tone. Mental relaxation is likewise difficult in certain definite groups of subjects, such as may be designated as neurasthenics, although by a few repeti-

tions of the test it is usually obtained. There is another group also in which relaxation seems even more difficult, and that is in patients who have hypertension; sometimes the metabolism is persistently elevated in this group, and a close clinical study of these patients seems to indicate that the central nervous system cannot readily "let go" and permit of the normal resting dissociation between it and the various tissues. Fear definitely elevates the metabolic rate. On the other hand certain patients seem to dissociate very readily, and low results may be obtained approaching that found in sleep especially in trained subjects.

3. Preliminary rest period. If the subject has not undergone severe muscular exercise (that is, in relation to capacity) we have found that twenty minutes is the minimal and forty minutes the maximal effective period of absolute rest needed to obtain the test under basal conditions. Severe exertion may require several hours' rest before the basal level is regained, and therefore should never be permitted before a test.

4. Normal temperature. Du Bois has shown that an elevation of the temperature 1°C. will, on the average, increase the metabolic rate by 10 per cent. In consequence it is necessary to take the temperature at the time of the metabolism test, in order to be sure no febrile condition is present. Even if the temperature is normal at the time of the test and yet during the day there is an elevation of the temperature as a result of an intermittent febrile reaction, we have found that this apparently influences somewhat the level of the metabolism. The subject must be very comfortably warm, otherwise, shivering or an invisible increased tensity of the muscles with an increase in heat production results in order to maintain the normal level of the body temperature.

It should be emphasized that these conditions are standard and arbitrary and not comparable to that obtained for instance in deep sleep or after prolonged starvation.

NORMAL STANDARDS FOR COMPARISON. Very early in the development of the science of metabolism Newton's law concerning the rate of cooling of various sized bodies suggested to Bergmann, in 1847, the possibility of finding a relationship between the rate of heat production and the surface area of the body. Meeh, in 1879, suggested the following formula for estimating the surface area of animals of similar shape: $S = KW^{\frac{2}{3}}$ where S = surface area in square centimeters, W = weight in grams and K a constant, used by Meeh as 12.3 for man. Rubner formulated a law to the effect that the fasting and

resting metabolism in animals of varying size and species is proportional to the surface area, and presented a series of constants, k, for different species to be substituted in Meeh's formula.

Doubt concerning the relationship of the heat production and the surface area arose largely from a discussion of the applicability of Newton's law of cooling, and whether or not this could control the rate of heat production. The conception of control has been abandoned, however, by nearly all adherents of the applicability of the surface area law, and in recent discussions has been mentioned only by those opposing the method.

Although not previously definitely formulated there has, in its stead, sprung up the conception that the surface area is more closely proportional to the active protoplasmic mass of the body, and therefore to the rate of heat production than any other available biometric measurement.

In 1915 and 1916 Du Bois and Du Bois working under the scientific leadership of Lusk published a series of papers in which they developed a new and more accurate formula for the surface area.

$$A = W^{0.425} \times H^{0.725} \times 71.84$$

Where A is the surface area in square centimeters, W is the weight in kilograms and H the height in centimeters.

Simultaneously Du Bois and his associates determined the normal standard of heat production for each square meter of body surface for varying ages and for sex, and published a smooth curve and table expressing their results for the factors of age and sex (table 1), to be used with the factors for height and weight combined in their surface area formula.

Harris and Benedict, in 1918, presented a most extensive biometric analysis of human basal metabolism from which they found that a very close prediction of the daily heat production of a subject can be made by use of multiple regression equations:

$$\text{For men, } h = 66.4730 + 13.7516w + 5.0033s - 6.7550a$$
$$\text{For women, } h = 655.0955 + 9.5634w + 1.8496s - 4.6756a$$

where h is the total heat production for twenty-four hours, w is the weight in kilograms, s is the stature in centimeters and a is the age in years. These equations have been tabulated for convenient use so that they may be readily used as standards of comparison.

Dreyer has published a series of formulas for predicting the normal heat production on the basis of weight and age in which the weight to

be used is recommended as the subjects' theoretic weight calculated from the trunk length and chest circumference. Stoner has recently prepared an extensive table, so that the values of Dreyer's formulas based on weight and age may be readily obtained. Dreyer's own tables for getting the theoretically correct weight of the subject must be used in conjunction with Stoner's table. Stoner finds that these formulas give basal metabolic rates almost identical with those of Harris and Benedict, but he does not give data which lead one to expect them to have a greater average accuracy. Means and Woodwell, in a careful comparison of the three methods, Du Bois, Harris and Benedict, and Dreyer, showed that the two latter were no more accurate than the Du Bois, and therefore recommended that the Du Bois method be continued since it already is in common use. Certainly unless one goes to the trouble of using the theoretic weight of the subject there is no point in using Dreyer's tables and it would be very confusing to use his method incompletely as recommended by Stoner. In normal individuals the use of actual weight as suggested by Stoner instead of the theoretic weight will not make much difference, because in such individuals both the theoretic and actual weights are nearly alike. On the other hand, clinically, we constantly deal with individuals markedly over or under weight, and in such instances are in most need of exactness, therefore a careless and ill-thought out use of standards may seriously harm the individual patient.

Our views in favor of the Du Bois standards for adults have recently been published. It seems best, therefore, to present here the very critical opinion of Krogh which appeared recently but may be overlooked as it is incorporated in a paper dealing largely with apparatus.

> Having obtained a reliable value for the standard metabolism of a patient we have to face the difficult problem of comparing it with the normal. The splendid researches of a number of investigators, and especially of Benedict and his co-workers at the Nutrition Laboratory, have shown conclusively that the standard metabolism of a normal person is not simply proportional to his weight or to his "surface" but depends on a sum of factors, among which the sex, age, weight and height have been recognized as being of paramount importance. There are at present at the disposal of clinicians two different systems for computing or "predicting" the normal standard metabolism of persons on the basis of these 4 factors, the Du Bois "surface" chart and simple table and the elaborate Harris-Benedict prediction tables. Each of these systems has certain advantages, and the choice is, therefore, exceedingly difficult.
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> According to Boothby and Sandiford the metabolism calculated for the same person by the methods of Du Bois and Harris-Benedict, respectively, shows on the whole a satisfactory agreement, but the metabolic rate calculated from the

Harris-Benedict tables are on an average about 4 per cent lower than those found by the Du Bois formula. The difference appears to be slightly larger (4.5 per cent) for men than for women (3.9 per cent), but this may be accidental. When the rules of procedure given above are followed closely, the lowest possible values will be obtained for the metabolism, and determinations made on normal subjects will probably agree more closely with the Harris-Benedict than with the Du Bois predictions. This holds good only with certain reservations, however.

The Harris-Benedict prediction-tables are calculated from two formulas worked out by statistical methods from the entire material of determinations. The formulas run:

For males $h = 66.4730 + 13.7516w + 5.0033s - 6.7550a$

For females $h = 655.0955 + 9.5634w + 1.8496s - 4.6756a$

where h = "basal" heat production for 24 hours, w = weight in kilos, s = height in cm., and a = age in years.

TABLE 1

Du Bois normal standards

Calories for each square meter of body surface each hour to be used with the height-weight formula: $A = W^{0.425} \times H^{0.725} \times 71.84$

AGE	MALES	FEMALES
years		
14–15	46.0	43.0
16–17	43.0	40.0
18–19	41.0	38.0
20–29	39.5	37.0
30–39	39.5	36.5
40–49	38.5	36.0
50–59	37.5	35.0
60–69	36.5	34.0
70–79	35.5	33.0

It is evident that these formulas are purely statistical and can have nothing to do with the biological laws which must be supposed to govern the relations between the metabolism and sex, weight, height, and age, respectively. In biological formulas we could not have a fundamental constant of 66 for men and about 10 times as high (655) for women and we could not have the influence of height about 3 times as large for men as for women. Above all we could not have the weight, height, and age of a subject represented as having simply additive influence upon the metabolism and we could not, as pointed out by Boothby and Sandiford, accept the influence of age to be a simple straight-line function of the age, and totally independent of the size of the subject.

The fact that these formulas are of a statistical nature and represent the influence of each factor simply as proportional to the numerical value of that factor implies that the predictions based on them must be very reliable for subjects who have weights, heights, and ages in the neighborhood of the average weight, height, and age for the total number of individuals upon whom the deter-

minations have been made, but will be increasingly unreliable the more the subjects differ from that average.

The Du Bois method of calculating the normal metabolism of a subject from the sex, age, weight and height, is based on an estimation of the surface area and assumes that for a given sex and age the metabolism is proportional to this area. While it must be admitted that the metabolism has probably nothing whatever to do with conditions governing loss of heat or with the outer surface of the body as such, the researches, especially of Dreyer and his collaborators point very clearly to the existence of a definite proportionality between the bodily functions and the nth power of the weight, w^n, where n is approximately $\frac{2}{3}$ and represents also the relationship between surface and weight. However this may be, Boothby and Sandiford have shown that for persons of normal build the formulas of Harris-Benedict can be transformed into formulas for calculating "surfaces" which will agree very closely with those deduced from the Du Bois height-weight chart. The two methods, therefore, show a practical agreement regarding the factors for weight and height.

The age-factor is introduced by Du Bois by means of a small table giving the calories per square meter for the age intervals 14–16, 16–18, 18–20, 20–40, 40–50, 50–60, 60–70, and 70–80 years, according to experiments on groups of subjects. It would certainly be preferable to use a smoothed curve,[1] from which the age-factor could be read off, but it must be admitted that the Du Bois way of introducing the age factor is in much better agreement with biological principles than the Harris-Benedict formula.

Boothby and Sandiford have published a series of determinations of standard metabolism on 102 normal subjects with their deviations from the Du Bois and the Harris-Benedict standards respectively. I find by a calculation of these results: $100\,\frac{M_o - M_{D\text{-}B.}}{M_{D.B.}} = 0.7 \pm 5.84$ per cent and $100\,\frac{M_o - M_{H.-B}}{M_{H.-B.}} = 4.8 \pm$ 6.06 per cent meaning that the observed metabolisms, $M_{o,}$ are on an average 0.7 per cent higher than those calculated by the Du Bois method, $M_{D.B.}$ and 4.8 per cent higher than the Harris-Benedict standards, $M_{H.-B.}$ while the dispersion (standard deviation) of the results is respectively 5.8 and 6.1 per cent, a difference which is certainly insignificant. A large number of these subjects had only one metabolic rate determination which will certainly on an average give a result which is too high on account of the "nervousness" of the subjects, and it is no cause for surprise, therefore, that the average results are higher than those calculated on either basis.

In conclusion the Harris-Benedict prediction-tables for the "basal" metabolism are very reliable for average subjects but, owing to their statistical nature, less so for persons of exceptional build or higher age. The Du Bois method and table for computing the basal metabolism give results which are on an average too high (about 4 per cent or more) but they are less likely to fail when used on exceptional subjects.

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[1] Du Bois in his original paper published such a curve which has been generally overlooked. A tabular form is however much more convenient for general use.

Joslin has published a most instructive monograph on the metabolism in 113 patients with diabetes, the technical work being done under the auspices of the Nutrition Laboratory. We have used his data and re-calculated the basal metabolic rate by the Du Bois standards for surface area to compare with his results expressed on the basis of the Harris and Benedict standards. Averaging the determinations for all cases except the six based on the girl-scout standards, we find that the average basal metabolic rate, according to the Harris and Benedict standards, is −3.4 per cent and by the Du Bois −7.7 per cent which apparently justifies Joslin's contention that there is little significant difference between the two standards. If the twelve subjects (excluding two boys) which have a variation between the two standards of ten points or over, are grouped and studied, it is interesting to note that they are all males between the ages of 35 and 72, and that they are markedly under the actuary standards of weight for their height and age. Reference to chart 3 of Boothby and Sandiford's paper, on comparison of standards, shows that discrepancies in the basal metabolic rate calculated by the two methods will occur with increasing age if the subjects are underweight, due to the Harris and Benedict method of introducing the age factor as a simple straight-line function of the age totally independent of the size of the subject. This is more marked in their formula for men than for women, because the age is multiplied by the factor −6.8 for men and by the factor −4.7 for women.

If we confine our attention temporarily to the height-weight factors of both methods we find in Joslin's series two subjects in whom the discrepancy between the Harris and Benedict and Du Bois height-weight factors, expressed in surface area, exceeded 10 per cent. One of these was a male 168 cm. in height and 101 kg. in weight and the other a female 141 cm. in height and between 32.2 and 25.7 kg. in weight. In both of these subjects variations between the two methods of determining the surface area are to be expected, as shown by Boothby and Sandiford in chart 2 of their surface area paper, because the male subject is rather short and heavy and the female subject is very short and light. There are eight other female subjects in Joslin's series which give variations from Du Bois' surface area between 6 and 10 per cent, which fall in the part of the chart indicating a slight variation from low weight.

The above differences in the standards of Du Bois and of Harris and Benedict, have been stressed in order that those who use either

standard will know under what condition the results of one will agree with the other, and where variations are to be expected. They also serve as a concrete illustration of the reasons why, as Krogh points out, the Harris and Benedict standards are more likely to fail than the Du Bois, when applied to exceptional subjects, and that they will be increasingly unreliable the more the subjects differ from the average age, height and weight of those on whom the statistical study and standards were based.

Another important fact receives practical illustration in Joslin's book, namely, that the girl-scout data of Benedict and Hendry or the larger data of Benedict for the ages between twelve and eighteen years, which were obtained when the girls were asleep, are not applicable for clinical standards with girls of similar ages awake and incompletely relaxed. Benedict found on the average a 12 per cent increase in metabolism in his thirty-one day fasting man when the determinations were made on the respiration apparatus in the morning with the subject awake as compared with that obtained while asleep in the respiration calorimeter. This was a fully trained and experienced subject, so that the difference found would be much smaller than in young girls who are entirely or relatively untrained. A confusion of standards based on the condition of sleeping and waking must be carefully avoided. On the other hand, the children studied by Du Bois were somewhat restless during their confinement of several hours in the respiration calorimeter, thus accounting, in part, for the large difference in the standards of Du Bois, and of the girl-scout data of Benedict. In the former the subjects were awake, mentally alert and more or less restless, while in the latter they were asleep and relaxed both mentally and physically. For the determination of standards suitable for clinical application the heat production must be determined for short periods under conditions arbitrarily comparable to those suitable for clinical investigation, and not under some other set of conditions.

In 1916 Du Bois presented a curve of the metabolism for each square meter of body surface referred to age which showed, as compared with that of adults, a distinct rise in metabolism with decreasing age until infancy was reached, when there was a progressive sharp drop. The general trend of this curve has been confirmed by the results obtained by Benedict and Talbot, and especially by Talbot's more recent curves presented in 1921. Although Talbot's values for each square meter of body surface vary considerably from those of Du Bois for the various ages, the character and general trend of the curve are the same, and

leave little doubt with regard to its characteristic shape. On account of the much larger series of cases which Talbot has studied since the Du Bois curve was published, it seems probable that Talbot's curve is more reliable up to the age of six or eight years. Beyond that age Talbot has relatively few cases and after twelve years the curve is a frank projection. Apparently the latter part of Talbot's curve is influenced by the data of Benedict on the sleeping girl scouts, referred to by him although at that time unpublished, which possibly accounts, in part, for its rapid fall; on the other hand the curve of Du Bois falls less slowly than it should, because the heat production was elevated above the level of that due to being awake by restlessness on the part of his subjects.

Both Benedict and Talbot consider that the shape of the curve in infancy and childhood with the varying values for heat production for each square meter of body surface, is conclusive proof of the fallacy of the surface area law. Such a conclusion is justified if the underlying principle of that law were a control of the heat production by the surface area. As pointed out any such conception of the law has been abandoned and instead it is considered that the surface area is the most exact of any biometric measurements yet suggested which will indicate the probable relative values of active protoplasmic mass in different individuals with approximate accuracy. Such a conception is in harmony with the findings of Moulton, who has shown that in cattle the surface area is proportional to the total body nitrogen and therefore to the protoplasmic mass. Likewise, Dreyer has called attention to the correlation between the cross-section of the trachea and aorta and the surface area of the body; that is, these vary in proportion to the entire protoplasmic mass that must receive oxygen through these channels.

If all this is relatively true then great biologic significance can be attached to the shape of the curve of heat production according to surface area referred to age. It shows that during the active period of growth the heat production for each unit of active protoplasmic mass is increased proportional to the intensity of growth. The sharp drop in the curve between two years and birth can be accounted for consistently with our general knowledge of the effect on metabolism of the central nervous system, as illustrated in part by the variation in heat production between sleeping and waking and by the fact that, anatomically, the central nervous system is not completely developed until about the age of twelve or sixteen months. Up to this time the

muscles are flabby and without tone, and the muscular movements are so incoördinate that walking is impossible. Furthermore there are no psychic influences at this period to elevate the heat production. In this connection there is significance in the observation of Talbot that the metabolism of older infants is higher than of younger infants of the same size. Also the fact that the metabolism of premature infants is lower than that of normal infants is what would be expected and predicted from a prolongation backward of the curve of calories for each square meter of body surface. The early part of the curve, therefore, is very instructive in indicating the development of the control of the nervous mechanism over the various organs and tissues of the body. The further facts that the heat production of the actively growing protoplasmic mass during the first month of infancy when practically uninfluenced by the nervous mechanism is between 700 and 800 calories for each square meter of body surface, and in Benedict's fasting man asleep the heat production of the non-growing protoplasmic mass varies between 560 and 660 calories for each square meter each twenty-four hours, illustrate roughly, the influence of growth on the heat production in the young, as contrasted to that needed for the maintenance in the adult. In both the psychic and nervous control is eliminated to a large extent, in the former by incomplete development and in the latter by sleep.

It is true that standards, which reveal such marked normal biologic variations in the intensity of the metabolism within a short time interval, like that described for surface area, are not of practical value in determining specific pathologic variations in individual infants. And since the curves of calories for each kilogram referred to age, minimize these normal influences in infancy, they may be more practical as an arbitrary reference during the first three years of life, as emphasized by Benedict and Talbot, and by Talbot. Furthermore, before the standards for older children can have much practical use clinically, they must be redetermined for short period experiments with the same type of apparatus and under conditions comparable to those used in the clinic.

Finally by laying aside all reference to Newton's law of cooling, and by frankly assuming that the law of surface area is, at the present time, the best available biometric measurement of the active protoplasmic mass of the individual, the line of thought and reasoning developed by Lusk, Du Bois and their students is in complete harmony with that of Benedict and his associates, and the threatened divergence in the

views of Benedict and Lusk fades away. On the evidence available all can agree that the main thought of both leaders is correct: Benedict, in his insistence on the fact that the rate of heat production is primarily dependent on the active protoplasmic mass (other factors being equal), and Lusk in his conviction that the surface area law expresses a fundamental biologic relationship, as it must if it represents a practical method of measuring the active protoplasmic mass.

REGULATION OF THE BODY TEMPERATURE. Lusk has devoted a chapter in his book to the question of the variation in heat production to maintain the body temperature. Grafe likewise gives a comprehensive review of the subject with references to the original articles. Rubner introduced two terms "physical regulation of the temperature" and "chemical regulation of the temperature." By the former he indicated the ability of the animal to vary the loss of heat by modifying the amount of blood to the skin and the quantity of sweat, etc., which need not be discussed here. By "chemical regulation" he indicated the increase in heat production above the basal caused by a lowering of the external temperature below the point at which the physical regulation mentioned above is able to maintain the body temperature normal without an increase in heat production. There has been some contention as to whether or not this increased heat production arose from increased muscular contractions, like shivering or an elevation in the rate of heat production of the non-contractible tissues. The weight of experimental evidence is decidedly in favor of muscular contractions or increased muscular tonus as the main if not the entire source of this extra heat. In experimental work on the effect of various substances on the heat production, as Rubner pointed out, the subject must be kept at such a temperature that any chemical regulation due to shivering is not brought into play. For example, the extra heat production caused by food reduces the necessity for shivering, and in consequence under these circumstances the specific dynamic action of food is masked. Erroneous interpretations on the effect of food on metabolism will be made unless the subject is kept at the optimal temperature for basal metabolic determinations. As pointed out in our discussion on standards, at the present stage of knowledge there is insufficient evidence to prove that the basal heat production is in any causal way dependent on, or controlled by, the surface area of the body in the sense of Newton's law of cooling.

The experiments of Du Bois show that an elevation of the temperature in febrile diseases and following the intravenous injection of foreign

proteins, produces a rise in the basal metabolic rate of approximately 10 per cent for each degree centigrade above the normal temperature. He found a marked similarity in the magnitude of the reaction in the various fevers, although he pointed out that most of the patients whose metabolism was very high for the degree of temperature had typhoid or malaria with a high level of protein metabolism, and that most of those with low metabolism were tuberculous with low protein metabolism. Although the increased protein metabolism seems to be a factor of some importance Du Bois believes that it is outweighed by another and simpler factor, and asserts that this surprising uniformity of results suggests that we are dealing with the law of physical chemistry enunciated by Van't Hoff, which may be expressed "with a rise in temperature of 10 degrees centigrade the velocity of chemical reactions increases between two and three times." Van't Hoff and Kanitz gave several instances in plant and animal physiology in which this law apparently held, and Zuntz mentioned rather indefinitely the possibility of its general applicability to metabolism. It is Du Bois, however, who presents substantial evidence that the law holds in the diseased human body.

SPECIFIC DYNAMIC ACTION OF FOODS.- Lavoisier recognized that the heat production was increased after the ingestion of food, and since that time many investigators have been interested in this subject. Rubner's monograph on "Die Gesetze des Energieverbrauchs bei der Ernährung" is largely devoted to its elucidation. One of the main problems investigated by Lusk in his work on animal calorimetry was a most careful and detailed study of the facts and theories concerned in this interesting and most fundamental phenomenon. As Lusk is the last investigator who has carried out more than a few scattering and as yet uncorrelated observations on this problem, and as future investigations must take his data and conclusion as a starting point, we cannot do better than to quote from his book a summary of the ways in which he conceives that the basal metabolism may be acted on by food:

1. *Amino-acid stimulation.* In amino-acid stimulation some oxy- or keto-acids derived from protein metabolism stimulate the cells to a higher level of oxidative activity.

2. *Fat plethora.* Here an influx of fat from the gut increases the heat production at the expense of the fat itself. When fat is oxidized two carbon atoms are broken from the chain together. What form this cleavage takes is not known; it is usually pictured as productive of acetic acid. If palmitic acid broke up by successive oxidations into acetic acid radicles, one could write the following reaction:

$$C_{16}H_{32}O_2 + 140 = 8\ C_2H_4O_2$$

Palmitic acid Acetic acid

1 gram = 1.348 grams

9353 calories = 4706 calories

Such a reaction involves 50 per cent loss of heat. Perhaps the energy imparted to the cell in fat metabolism is derived from a twofold source, acetic acid and the oxidation at the B-carbon atom of the fatty acid; or perhaps a substance more highly explosive than acetic acid is set free as the result of B-oxidation. In any event one may conceive of the oxidation of fat as being in the nature of successive ultra-micro explosions, which act as power for the machinery of the cells.

3. *Carbohydrate plethora.* Here an influx of carbohydrate from the intestine increases the heat production. When these enter the circulation alone they are oxidized to the exclusion of fat. It appears certain that the intermediary metabolites of glucose and fructose are far more readily oxidizable than fat, and on this account, when they are present, they satisfy the energy requirements of the cell, and the fat is not attacked. If glucose breaks up into methyl-glyoxal, and this into acetaldehyd and formic acid, the reaction would be as follows:

$C_6H_{12}O_6 - 2H_2O$	$\rightarrow$	$2C_3H_4O_2 + 2H_2O$	$\rightarrow$	$2CH_3CHO + 2HCOOH$
Glucose		Methyl-glyoxal		Acetaldehyd
1 gram				0.4889 gram
3.762 calories				3.098 calories

According to this computation, and assuming that hydrolytic reactions are accomplished without thermal changes, 79 per cent of the energy liberated in carbohydrate metabolism would be derived from acetaldehyd, and 21 per cent from formic acid.

There is no indication of a physiologic separation of these two varieties of energy. It happens frequently that with the cessation of glucose absorption the respiratory quotient remains at 1.00, indicating that carbohydrate is still the essential food, and yet the metabolism has fallen to the basal level. One must, therefore, conclude that the metabolism increases only in the presence of a plethora of dissociated fragments of sugar. The metabolism may rise to a certain height which is not transcended, and an excess of metabolites above this level may be coverted into fat with scarcely any energy loss.

4. *Carbohydrate and fat plethora.* Here there is a summation of effect. It seems as though that part of the cell-mechanism which is susceptible to fat metabolism when fat is present in excess, is not inhibited from metabolizing such surplus fat, even in the presence of carbohydrate.

5. *Amino-acid stimulation and carbohydrate and fat plethora.* Simultaneous ingestion of an amino-acid and carbohydrate acts in such a manner as to suggest that the increase in metabolism due to carbohydrate plethora is essentially independent of that due to the chemical stimulus of amino-acids. Also, when an amino-acid is given with glucose at the height of fat metabolism (four hours after fat ingestion), the increase in heat production is nearly one, amounting to a summation of the three influences.

One may, therefore, conclude that the influence of food on the quiet resting cell under these circumstances is on three independent mechanisms within the cell:

1. A mechanism which is receptive to a chemical stimulus derived from the metabolism of such amino acids as glycocoll and alanin.

2. A mechanism of carbohydrate plethora which allows the metabolism of carbohydrate up to the limits imposed by "self-regulation."

3. A mechanism capable of receiving power from that quota of fat which, when in excess, increases the heat production of the cell.

Du Bois, Means, Benedict, Joslin, Boothby and others, have made a few observations on the specific dynamic action of protein, carbohydrate and fat in various diseases, and as yet no definitely consistent variations have been obtained in the magnitude of the reaction from that found in animals or normal human subjects. The entire problem, however, on account of its fundamental character deserves still further investigation directed to a more extended determination of the quantitative and time relationships. Furthermore this evidence, when obtained, must be correlated with such results as those of Krogh and Lindhard, which indicate the conversion of fat into carbohydrate as well as the reverse reaction, and which if true cause some loss of energy and may limit the conditions under which the isodynamic law holds. This law was broadly formulated by Rubner as follows: Foodstuffs may, under given conditions, replace each other in accordance with their heat-producing value.

FASTING AND UNDER-NUTRITION. Three large monographs and several subsidary papers by Benedict and his associates, devoted to the study of the effect of fasting and under-nutrition have appeared from the Carnegie Nutrition Laboratory. All three contain an immense amount of most valuble data, as well as references to the literature on the subject, and it is impossible unfortunately to refer to more than a few of the points which deal directly with the subject of basal metabolism.

Benedict found that during a thirty-one day fast there is a definite, but gradual reduction in the heat production, when compared on the basis of the amount of heat produced during sleep, that in the morning when awake, the total calories for each twenty-four hours, or calories for each square meter of body surface; he found, however, little significant change if compared on the basis of calories for each kilogram of body weight. He concluded that a decrease during fasting amounting to 28 per cent for each square meter of body surface can be interpreted only as proof of the inapplicability of the surface area law to subjects with widely varying states of nutrition, and furthermore maintains that a standard value obtained with robust, normal subjects cannot be used as a comparison for emaciated diabetic patients. However he, himself, cannot stress this point because Joslin has recently from

Benedict's laboratory compared diabetic patients with the Harris and Benedict standards. Benedict is correct in his statement that the evidence of a decrease in metabolism in starvation indicates that the surface area of the body does not control the rate of heat production; however on that ground he is not justified in ignoring the biologic significance of the important fact that the heat production of individuals of like age and sex under standard conditions is proportional to the body surface. The evidence of Benedict, Miles, Roth and Smith, also shows that healthy men kept on a reduced diet for several months showed a reduction in the level of the basal metabolic rate. They likewise refer to a similar reduction below normal of diabetic patients studied by Joslin who have been kept for a considerable time on a sub-maintenance and starvation diet. Wilder, Boothby and Beeler have reported a considerable series of observations on thirty-one diabetic patients who had basal metabolic rates below normal. Those who had uncomplicated diabetes and body weights 20 per cent or more below the standard weight showed a depression in the basal metabolic rate of -11 to -32 per cent on the basis of the Du Bois standards for surface area; the degree of depression was roughly proportional to the degree of loss of weight below the standard.

As pointed out in the section on standards the surface area seems to be the most accurate biologic measurement at present available for estimating the relative mass of active protoplasmic tissue in various individuals of the same as well as of different species. The fact that there is a decrease of 28 per cent in the rate of heat production for each square meter of body surface due to prolonged starvation, and by inference a proportional decrease in the rate of metabolism of a unit of active protoplasmic mass is of the highest biologic significance, and on teleological grounds is to be expected as a means of conserving the stored food and prolonging life. As Benedict suggests, this decrease in metabolism may be due to a loss of nitrogen or protoplasmic tissue. While this is quite an important factor it cannot, as indicated below, account for the entire amount of the observed reduction. The loss of nitrogen in Benedict's fasting man was a total of 277 grams. Moulton has shown that in beef cattle the total amount of nitrogen in the body can be estimated by the formula: $S = N^{\frac{2}{3}}$, where S is the surface area in square meters and N the nitrogen in kilograms. If this formula holds even approximately for man the total amount of nitrogen in the human adult body with a surface area of 1.71 square meters would be in the region of 2.24 kilograms. Therefore, the loss of protoplasmic mass

or nitrogenous tissue during the fast can be estimated with some degree of assurance as approximately 12 per cent. The decrease in surface area during the fast was from 1.71 to 1.53 square meters (photographic method) or a decrease of 11 per cent which takes care of the above estimated reduction of nitrogenous tissue and leaves, therefore, nearly all of the observed reduction of 28 per cent in heat production still to be accounted for. A similar calculation for the group of undernourished individuals of Benedict, Miles, Roth and Smith gives comparable results. Therefore it is reasonable to assume that the heat production may be predicted with considerable accuracy on the basis of variations in the surface area due to changes in weight from a parallel loss of body fat and nitrogenous substances. In consequence the large decreases from starvation or undernutrition found in the heat production for each square meter of body surface probably represents quite accurately a corresponding lessening under such conditions of the heat production of a unit mass of protoplasmic tissue. This evidence supports the idea that under-nutrition and starvation cause a decrease in the rate of heat production of a unit mass of protoplasmic tissue.

The fact discovered by Benedict and Talbot that there is an increased metabolism in children with malnutrition is at first sight confusing. In several articles they contrast the increased metabolism from malnutrition in infancy with the above noted finding of a decreased metabolism in under-nutrition in adults and conclude that the effect of under-nutrition is reversed in the infant as compared with that found in the adult. An interpretation for infancy that reverses a well-established biologic fact for adults should not be adopted without close scrutiny to see if the facts as found cannot be as readily interpreted without such reversal of a fundamental principle. The cases studied by Talbot were not infants who were normal with the exception of such changes as might be expected to occur from a premeditated withholding of food such as occurred in the case of Benedict's fasting man, or the group of voluntary restricted workers of Benedict, Miles, Roth and Smith. Talbot's infants were sick, they could not take, digest or assimilate their food until the disease itself was relieved. Is it not reasonable to infer that the disease itself very possibly influenced the metabolism? Talbot himself says:

> Severe infantile malnutrition has received many names, such as infantile atrophy, marasmus, atrophy, athrepsia and decomposition. It does not represent a definite disease entity, nor is there any clear description of exactly what the

condition is. It is found in all degrees of severity and it is often difficult to state just when it commences. It is usually associated with symptoms of indigestion, either vomiting or diarrhoea, or both, and a loss in weight. Some infection, such as pyelitis or bronchitis, often complicates the condition. When most pronounced, the infant is very much wasted, the skin hangs in folds, and is of a gray pallor, the hands and feet tend to be cold, and toward the end the temperature becomes subnormal and has to be maintained by external heat. Metabolism experiments show that the infant is frequently unable to absorb the food given him. There is no common pathologic basis for the symptoms found in this condition.

Evidence obtained from sick infants emaciated as the result of disease of unknown etiology certainly cannot be used as the basis of argument that fasting or undernutrition from insufficient food produces a reversed effect in healthy infants as opposed to healthy adults. On the contrary it probably means that the factor causing the disease likewise causes, either directly or indirectly, an increase in the rate of metabolism.

Kunde has recently published a study of the oxygen consumption on herself from April, 1921, to February 28, 1922; determinations were made almost daily throughout this time, and between September 14 and 28 was a fifteen day period of complete fasting. A few determinations were made on a male subject before, during and after a fourteen day fast. On herself, Kunde found a distinct rise in the metabolism for each square meter of body surface during the first few days of the fast, which subsequently decreased toward the end but still remained above the rate of metabolism before the fast. The other subject likewise showed a rise in metabolism during the first few days followed, however, by a decrease below that preceding the fast. Both these subjects carried on their daily work, the one her laboratory duties as well as going back and forth from her residence and the other his daily work in a flour mill. A very much larger amount of work during the day was performed by these persons than was done in the experiment on Benedict's fasting man. If the experiments of Hill and his associates and of Meyerhoff are correct as to muscular work being due to a characteristic reaction of a carbohydrate-like substance, and if the work of Krogh and Lindhard on the loss of energy in the formation (both during rest and work) of this substance from fat is confirmed, then the explanation of the findings of Kunde may be interpreted in part as the waste of energy from the utilization of fat in work. In addition the exertion of coming to the laboratory and the effort of carrying out a considerable part of the experimental work might readily produce during the fasting period a relatively greater degree of fatigue than during the non-fasting periods

on account of the progressively increasing weakness, and as a result a complete basal condition would not be obtained by the ordinary preliminary rest period. In regard to the effect on the basal heat production in dogs from prolonged fasting we have calculated from her data for the last five days preceding the fast and the last five days of the fast that no appreciable change in metabolism for each square meter of body surface occurred in dog 1, while there was a 9 per cent reduction in dog 2 and an 18 per cent reduction in dog 3. We do not believe that her data justify the conclusion that "a fast of 41 days in dogs and 15 days in man causes no appreciable lowering of the basal metabolic rate during the fasting period."

Lusk in his review on the physiological effects of under-nutrition calls attention to the experiments of Zuntz and Loewy on themselves during the war. Loewy lost gradually in weight during 1916 and the early part of 1917 and simultaneously his heat production fell, but in July 1917 when he had lost 22 per cent of his former weight and when the total calories and protein of his diet were low, his heat production suddenly rose again to a level above that observed in 1914. On the day of observation the nitrogen of the food was 8 grams and that of the urine 17 grams. Zuntz and Loewy compared this result with precisely similar observations made by Zuntz on an undernourished, but otherwise normal dog. As Lusk points out, this may explain in certain instances the high metabolism sometimes found in extremely emaciated diabetics.

THE EFFECT OF WORK. The literature is rich on the cost of muscular work, and has been very efficiently reviewed by Grafe. The recent investigations of Hill and Hopkins and their associates, have opened up an entirely new phase of the problem, especially with regard to the causes of the increased heat production after work. Benedict and Cathcart show, however, that the oxygen consumption of light work is approximately the same whether or not it is preceded by severe work, in spite of the fact that there is demonstrable a definite after-effect on the resting metabolism. They developed a series of formulas which appears to indicate that the efficiency is greater after work, corresponding to a "warming up" process. In the light of Hill's investigations it seems possible that this after-effect is part of the aerobic or recuperative phase of muscular reaction and not to a change in efficiency. The figures of Benedict and Cathcart show that if this is true the aerobic part of the reaction proceeds more or less simultaneously with the anaerobic phase in the intact working organism because the amount of the after-effect

is only a small proportion of the work performed per minute and therefore merely represents the rapid although gradual ending of the reaction. In this sense, then, comparatively little change in the base-lines for work preceding and following severe work is to be anticipated and "second wind" may be considered as the development of the reversed anabolic reaction up to the speed proportional to the catabolic reaction. That an appreciable time is required for this reversed chemical reaction, first, to get under way and second, to stop, is not surprising.

Benedict and Murschhauser show that up to 20 kilometers there is no definitely uniform influence of prolonged walking on the efficiency for horizontal movement, and conclude that up to 80 meters each minute walking may be done for many kilometers without affecting the efficiency of the body for locomotion in a horizontal direction.

Smith, in an extensive study from the Nutrition Laboratory on walking, calculates that the amount of glycogen consumed during one hour of walking on the basis of an oxygen consumption of 2,500 cc. each minute, and a respiratory quotient of 0.90, would be somewhat more than 100 grams, or more than one-fourth of the amount believed to be present normally in the body. He confirmed the finding of Zuntz and Schumberg, and others, that the respiratory quotient is lowered after an interval of rest in relation to the respiratory quotients obtained both before and during exercise. Smith finds that the metabolism with the subject standing represents an increase of 12 per cent compared to that obtained with the subject prone. Using the standing requirements as a basis he finds that the increase in energy expended in horizontal walking over the energy output for the standing position varied for the eight subjects from 0.454 to 0.618 gram-calorie for each horizontal kilogrammeter. He also presents a curve showing that the efficiency for each horizontal kilogrammeter is greatest at about 2800 meters each hour, and that there is a decrease in efficiency both above and below this optimum rate of walking. In the transition from standing to grade walking Smith finds that in most cases the adjustment of oxygen consumption and other factors is nearly complete by the end of the third minute, and that the larger part of the adjustment has occurred within the first thirty seconds. The recovery after exercise is, as was also found by previous observers, not so prompt and the after-effects may persist for some time.

The cost of work, measured by determining the respiratory metabolism while walking on a treadmill at an approximately constant rate of 800 meters each hour, has been determined by Plummer and Boothby,

and by Boothby and Sandiford on five normal subjects, on eighteen patients having miscellaneous diseases, and on fourteen patients having either exophthalmic goiter or adenomatous goiter with hyperthyroidism. All persons in the various groups were studied under comparable conditions. Their figures however cannot be compared directly with those of Smith quoted above because the base line is different and the rate of walking less than one-third that found to be most efficient by Smith. In certain unpublished experiments from this laboratory on normal subjects, with speeds around 3,000 meters each hour, values were found quite similar to Smith's and confirm the finding of a decrease in efficiency at slow rates of walking. Boothby and Sandiford were principally concerned about the comparative efficiency in their subjects of all muscular work, and not that due to horizontal propulsion as differentiated from that necessary to maintain the erect position and, therefore, they chose as their base line the standard basal

TABLE 2

*Cost of work expressed as net gram calories for each horizontal kilogram-meter**

DIAGNOSIS	SUBJECTS	AVERAGE BASAL METABOLIC RATE	NET GRAM CALORIES FOR EACH HORIZONTAL KILOGRAMMETER	DEVIATION FROM NORMAL OF 1.20
Normal	5	−10	1.20	
Diabetes mellitus	4	−13	1.09	−10
Myelogenous leukemia	2	+20	1.10	−8
Anorexia nervosa	1	−22	1.12	−7
Lymphatic leukemia	1	+65	1.19	−1
Addison's disease	1	−8	1.27	+6
Chronic nervous exhaustion	1	+5	1.34	+12
Myxedema	5	−28	1.36	+13
Hypertension	3	+4	1.43	+19
Carcinoma of the thyroid	1	+43	2.31	+93
Exophthalmic goiter	12	+52	2.24	+87
Adenoma with hyperthyroidism	1	+47	2.86	+138

* Calculated as follows:

$$\frac{(\text{T.C.H.W.} - \text{T.C.H.B.}) \times 1000}{\text{M.H} \times \text{Wt.}} = \text{Net gram calories for each horizontal kilogrammeter}$$

where T.C.H.W. = total calories per hour at work, T.C.H.B. = total calories per hour in bed, M H. = meters per hour, Wt. = walking weight of subject in kilograms and the constant 1000 to convert large calories into gram calories.

The basal heat production in bed under standard conditions was chosen for the base-line because our interest lay in the net cost of all work, including that for maintaining the upright position.

condition with the subject prone. The results obtained, expressed as net gram-calories for each horizontal kilogrammeter, are summarized in tables 2 and 3.

TABLE 3

Cost of work in a case of exophthalmic goiter (S.H., a man, aged twenty-five years and a college athlete)

DATE	WEIGHT	RESPIRATORY QUOTIENT	TOTAL CALORIES FOR EACH HOUR	METABOLIC AND BASAL METABOLIC RATE	STEPS FOR EACH MINUTE	HORIZONTAL METERS FOR EACH HOUR	NET GRAM CALORIES FOR EACH HORIZONTAL KILOGRAMMETER
	kgm.			*per cent*			
12/ 7/22	70.2	0.81	115	+59			
12/ 9/22	70.0	0.78	122	+71			
12/11/22	69.0	0.79	114	+59			
		0.81	232	+221	30	791	2.16
12/12/22	70.5	0.74	104	+44			
		0.86	217	+199	27	787	2.04
		0.78	219	+201	24	787	2.07
12/13/22	69.9	0.79	108	+51			
12/14/22	Thyroidectomy						
12/18/22	66.1	0.74	104	+48			
12/20/22	65.8	0.76	88.1	+25			
		0.76	179	+156	26	797	1.73
		0.74	180	+156	24	800	1.75
12/21/22	66.8	0.74	87.6	+24			
12/22/22	67.2	0.79	83.6	+18			
		0.74	179	+152	24	802	1.77
12/23/22	67.5	0.82	87.0	+22			
		0.78	171	+140	25	802	1.55
12/26/22	68.5	0.85	80.9	+12			
		0.81	161	+124	24	791	1.48
12/27/22	69.0	0.82	79.3	+10			
		0.89	156	+116	25	793	1.40
12/28/22	69.9	0.84	76.6	+6			
		0.87	156	+116	24	797	1.43
12/29/22	69.5	0.90	78.1	+8			
		0.84	159	+121	24	797	1.46
12/30/22	69.6	0.89	77.3	+7			
		0.82	151	+108	24	785	1.35

The results show that patients with exophthalmic goiter and adenomatous goiter with hyperthyroidism require nearly twice as much energy for a given piece of work as do normal persons or even patients with other diseases. Furthermore, the determinations made on one patient before and after thyroidectomy (table 3), illustrate very conclusively the improve-

ment in muscular efficiency, as the patient recovers from the disease, as evidenced by a decrease in the basal metabolic rate to the normal range.

The experiments of Krogh and Lindhard, already referred to, reopen the question as to the possibility of fat being converted into a carbohydrate-like body. In the three best series of their experiments they find the net expenditure of energy for each calorie for technical work varies from about 4.6 calories when fat alone is catabolized to about 4.1 calories when carbohydrate alone is catabolized. The waste energy from fat is 0.5 calorie or 11 per cent of the heat of combustion of the fat.

Pregnancy. Carpenter and Murlin reported three very interesting cases in which they showed that the extra metabolism of the pregnant woman at the culmination of pregnancy, due in part to the accessory structures as well as to the fetus, is just equalled by the metabolism of the mother and child taken together 3 to 10 days after delivery. The energy metabolism of the new-born child expressed per unit of weight found by subtracting the metabolism of mother alone from that of mother and child together, is two and a half times that of the mother. Expressed per unit of surface (Meeh's formula) the energy metabolism of the new-born child is not greater than that of the nursing mother but is higher than that found in eight women in complete sexual rest. These findings were in essential agreement with those previously found by Murlin in dogs, the most important of which was the evidence that the curve representing the energy production of both mother and offspring combined suffered no demonstrable deflection at birth.

The recent work of Root and Root shows a marked increase in the basal metabolism in the total 24 hour calories and in calories per kilogram during the latter months of pregnancy. However according to the usual method of determining the basal metabolic rate by both the Harris and Benedict and the Du Bois standards, the heat production throughout pregnancy remained except for one determination within normal limits. Any increase that may exist in the rate of heat production on the part of the mother, as distinct from the fetus, during the pregnancy is very small. We believe that at the present time not enough data are available to establish with certainty whether there is a slight increase or decrease of the order of 5 or 6 per cent in the metabolism of a unit mass of active maternal protoplasmic tissue because the variations found depend entirely upon the method of calculation adopted.

The marked increases in metabolism in pregnancy frequently reported in clinical literature contain such obvious discrepancies and evidence of improper technic that they deserve little or no consideration.

Menstruation. Ford, Snell and Rowntree found variations in the heat production in women which they believed, at the time, might be indicative of the influence of menstruation. Their curves, as well as those of Rowe and others who have reported similar findings, show that the changes in metabolism can be equally as well interpreted as accidental variations independent of the menstrual cycle. On the other hand Zuntz, Lusk, Gephart and Du Bois, Boothby and Sandiford, Blunt and Dye, Kunde and others, have been unable to find any evidence that the heat production is appreciably affected by the menstrual cycle. Wiltshire not only found that the basal metabolism was unaffected by menstruation, but that the cost of muscular work and the rate of recovery after work, remained the same. We have observed, not infrequently, in our laboratory a rise in the basal metabolic rate if the subject is in pain or distress whether of menstrual or other origin; a rise due to pain must be carefully excluded when considering the effect of menstruation itself on the rate of heat production.

Drugs. Loewy has presented an excellent summary of the older work on the effect of drugs on the heat production. Boothby and Rowntree on the basis of their experiments on 25 of the common drugs taken in conjunction with those reported in literature summarized the action of drugs on the basal metabolic rate as follows: The common drugs (not including preparations representing internal secretion) if administered in single, moderate doses do not demonstrably influence the basal metabolic rate. Caffein alkaloid in large doses seems to cause an elevation in the heat production (Means, Aub and Du Bois), but did not do so in the smaller doses used by Boothby and Rowntree. Iodin, or the iodids, if administered in a sufficient quantity for several days to patients with an adenomatous enlargement of the thyroid gland is apt to cause an elevation of the basal metabolic rate by converting the condition of adenomatous goiter without hyperthyroidism into the disease designated by Plummer as adenomatous goiter with hyperthyroidism (Snell, Ford and Rowntree). Iodin, in the form of Lugol's solution, nearly always causes a marked drop in the basal metabolic rate with coincident clinical improvement in patients with exophthalmic goiter (Plummer). Morphin in sufficient quantity to produce sleep lowers the heat production (Lusk).

Of the preparations representing the secretions or the active principles of the ductless glands, adrenalin has a definite and intensive calorigenic action with its maximum effect a few minutes after administration (Boothby and Sandiford). Insulin does not apparently possess a direct calorigenic action, although it causes an increase in the basal metabolic rate in the fasting individual if the blood sugar falls to a critical level associated with symptoms of hypoglycemia; this reaction, however, appears to be secondary to the mechanism which helps to stop the further progress of the fall in the blood sugar level (Boothby and Wilder) Thyroxin possesses a definite and quantitative calorigenic action which does not appear for several hours after administration, and reaches its maximum effect after several days; in the thyroidless person, this effect may not disappear for several weeks (Plummer and Boothby). No other ductless gland preparation, excepting those just mentioned, has yet been demonstrated to have either an immediate or a delayed influence on the basal metabolic rate (Aub).

In the clinical estimation of the basal metabolic rate neither adrenalin nor insulin should be given within several hours preceding the test. Although thyroxin does not cause an immediate rise in the metabolic rate, no test made subsequent to its administration at least for a period of several weeks, in a patient suspected of a metabolism below normal, can be assumed to represent the lowest metabolism of that individual. The administration of iodids does not affect the metabolism of a normal individual but may convert a case of adenomatous goiter without hyperthyroidism into one with hyperthyroidism, and the increased metabolism, when so produced, represents the intensity of the new syndrome. Aside from exceptions indicated here, it is unlikely that any of the ordinary drugs in doses used in clinical work will invalidate basal metabolic rate determinations.

For a more extended discussion of the action of thyroxin, adrenalin and insulin see the special sections devoted to them as well as those on adenomatous goiter with hyperthyroidism and exophthalmic goiter and on diabetes.

THE BASAL METABOLIC RATE IN DISEASES OTHER THAN THYROID. In this review it is impossible to treat in detail the various diseases and their possible influence on the basal metabolism. A study of table 4 which includes observations made in this laboratory on 1642 individuals with different diseases shows that except in thyroid disorders and in leukemia there is very little variation from normal of the basal metabolic level. The summary of table 4 which includes all cases studied in 1922,

TABLE 4

The basal metabolic rate in conditions not due to thyroid disorders in 1642 patients studied during 1922

DIAGNOSIS	CASES	PERCENTAGE RANGE							
		Below −20	−20 to −16	−15 to −11	−10 to +10	+11 to +15	+16 to +20	Above +20	−15 to +15
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Negative	76			6	84	9	1		99
Migraine	42			10	88	2			100
Chronic nervous exhaustion	191	1	1	5	86	6	1		97
Neurasthenia	210		1	11	80	6		2	97
Obesity	55	2	2	2	80	4	5	5	86
Asthenia	18		5	5	78	6	6		89
Essential hypertension	95		3	2	66	11	8	10	79
Cardiac neurosis	22				73	23	4		96
Cardiac miscellaneous	33			6	67	15	6	6	88
Endocarditis	48		2	2	65	21	4	6	88
Myocarditis	40	2		2	53	20	13	10	75
Pericarditis	5				80			20	80
Renal	80	2	2	14	69	4	1	8	87
Mental	28		4	4	82	7		3	93
Epilepsy	24		13	8	63	12		4	83
Gastro-intestinal	112	1	4	12	73	10			95
Gynecological	42	5		10	83		2		93
Malignancy	23	13	4	4	48	17	9	5	69
Dermatological	34			6	74	15	3	2	95
Pregnancy	29				79	10	7	4	89
Encephalitis	6			17	66	17			100
Dysphagia	14	57	14		29				29
Acromegaly	5				60		20	20	60
Hypopituitarism	14	7	15	14	50		7	7	64
Paget's disease	1				100				100
Addison's disease	12			17	67	8	8		92
Secondary anemia	25				96		4		96
Anemia, splenic and pernicious	36	3		3	53	8	19	14	64
Leucemia, lymphatic and myelogenous	15				27	7	6	60	34
Questionable ductless glands	1				100				100
Sclerosis of central nervous system; tabes	4				100				100
Diabetes	61	5	3	5	69	13	2	3	87
Arthritis	65	2	2	6	83	1	6		90
Miscellaneous, not thyroid	176	1	3	7	73	5	6	5	85
Total number of cases	1,642	1	2	7	74	8	4	4	89

except the thyroid diseases, shows that 74 per cent are within ± 10 per cent of the Du Bois normal standard and that 89 per cent are within ± 15 per cent of these standards. These figures agree with remarkable closeness to those in a previous report by us on the cases other than thyroid studied during 1917–1918–1919–1920 and 1921 which totalled 2417 and in which the metabolism was within ± 10 per cent in 77 per cent of the cases and within ± 15 per cent of the Du Bois standards in 90 per cent of the cases. Means and Burgess report similar figures on 1000 cases studied at the Massachusetts General Hospital.

These results are to be compared with those given in table 5 for 1689 patients with thyroid disorders studied in this laboratory during 1922 as well as the series previously reported by us of 6,197 cases with thyroid diseases studied between 1917 and 1921.

TABLE 5*

The basal metabolic rate in 1689 patients with thyroid disorders studied during 1922

DIAGNOSIS	CASES	PERCENTAGE RANGE									
		Above +75	+74 to +50	+49 to +21	+20 to +16	+15 to +11	+10 to −10	−11 to −15	−16 to −20	Below −20	Above +20
		per cent	per cent	per eent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Exophthalmic goiter	452	12	40	40	4	1	3				92
Recurrent exophthalmic goiter	56	11	16	61	3	2	5	2			88
Adenoma with hyperthyroidism	346	2	11	52	13	22					65
Recurrent adenoma with hyperthyroidism	11	18	9	36		37					63
Adenoma without hyperthyroidism	544						100				
Recurrent adenoma without hyperthyroidism	23						91	4	5		
Colloid goiter	140			1	1	7	81	4	5	1	1
Myxedema	12							17		83	
Postoperative myxedema	11							9	9	82	
Questionable hypothyroidism	42						26	19	24	31	
Cretinism	1						100				
Thyroiditis	27		4	41		7	44	4			45
Malignant thyroid	24		8	4	4	13	58		13		12
Total number of cases	1,689										

* The 3331 cases summarized in tables 4 and 5 represent all patients on whom tests were made during 1922 with the exception of 13 in which a positive diagnosis of the presence of hyperthyroidism was not reached.

In a consideration of the significance of tables 4 and 5, the probability of errors in the determination of the basal metabolic rate must be evaluated. We estimate

LEUKEMIA. That the basal metabolism is elevated in leukemia was first noted by Bohland. This fact has been fully confirmed by Magnus-Levy, by Grafe, by Murphy, Means and Aub, by Gunderson, by Lennox and Means and by observations in our laboratory. As yet sufficient cases are not available to determine if there is any marked and consistent difference in the level of the basal metabolism between the lymphatic and the myelogenous forms. The studies on the nitrogenous metabolism as reviewed by Grafe show that there is no definite increase in protein destruction; Lennox and Means have shown that a positive nitrogen balance can be maintained in acute leukemia on a diet containing 10 grams nitrogen a day. Murphy, Means and Aub showed that direct and indirect calorimetry gave total results which were almost identical. They also pointed out that the manifestation of an increased metabolism—the profuse sweating, the hot, flushed skin, the tachycardia, nervousness, tremor, etc.—so characteristic of exophthalmic goiter were absent or slight. We have also been interested in this difference which, however, is only slightly more marked than that between exophthalmic goiter and adenomatous goiter with hyperthyroidism.

that in routine work in our laboratory there is a material error in about 1 per cent of the determinations and in an additional 5 per cent of the tests slight errors occur, the result of which is to place the patient either in the next higher or lower group, as arranged in the table. In about one-third of the patients with thyroid disorders only one determination was made and approximately one-half the patients who had other diseases, not involving the thyroid, had only one rate.

If in any group 100 typical cases with unquestioned diagnosis are selected and on whom sufficient basal metabolic rate determinations are made so that all errors are excluded, such a series will usually show a 99 per cent agreement with the characteristic metabolism for that condition.

With a few exceptions the patients with exophthalmic goiter having basal metabolic rates below +20 per cent came under our observation during a period of remission.

The patients listed under recurrent exophthalmic goiter and recurrent adenoma, with and without hyperthyroidism, include those who had had a previous partial thyroidectomy before any metabolism studies had been made in our laboratory and in whom the question of the necessity for further operative treatment was under consideration.

A basal metabolic rate of +10 per cent has been taken arbitrarily as dividing patients with adenomatous goiter into the groups with and without hyperthyroidism. While all cases with basal metabolic rates below +10 per cent are unquestionably not hyperthyroid, it cannot be assumed that all those with basal metabolic rates slightly above +10 per cent are necessarily hyperthyroid.

Only rarely does a cretin come under our observation who has not had thyroid medication; therefore the results presented in the table cannot be considered as the average of a group of untreated cases. Furthermore, the normal standards for children are not yet as accurately established as are those for adults.

Grafe has suggested that the increased metabolism was due in large part to the rapid formation of new blood cells. In pregnancy the metabolism of the mother plus the fetus is only slightly increased and as the rate of formation of new cells must be very rapid it seems unlikely that the large increases found in leukemia can be explained entirely on that basis.

Whether or not in the future the query of Sandiford, Boothby and Giffin as to the possible influence on the level of the metabolism of the fact that the amino-acids of the blood are usually increased is of course at the present time mere speculation. The suggestion made by them will direct attention to the possibility that there may be present in the blood in this disease a hitherto unsuspected calorigenic agent of the catalytic type possibly containing an amino group which increases metabolism in some such way as adrenalin and thyroxin.

THYROXIN AND MYXEDEMA. To study the effect of thyroid secretion on the rate of heat production the completely myxedematous or thyroidless subject is preferred because it is theoretically probable that with a normal or hyperfunctioning thyroid the quantity of thyroid secretion produced by the gland might vary inversely either completely or in part with the amount of thyroxin introduced artificially; whether or not this possibility is true the evidence of the calorigenic action of thyroxin is much more easily interpreted if this variable is eliminated.

The literature on the effect of various thyroid preparations in man and animals has been extensively reviewed by Grafe. Inconsistent results have been reported due possibly in part to the cause mentioned, and in part to the fact that the thyroid was administered orally and the factor of incomplete absorption entered. Furthermore the ordinary preparations of thyroid are rarely if ever standardized according to the quantity of the active principle which they may contain.

Plummer and Boothby have reported a series of very carefully controlled experiments on sixty-nine cases of myxedema of the effect of the intravenous injection of different amounts of thyroxin. The basal metabolic rate in complete untreated myxedema may vary between −25 and −40 per cent, but is usually between −35 and −40 per cent.

As can be seen from the foregoing data and the calorigenic curve (fig. 1) the effect of 1 mgm. of thyroxin given intravenously is an increase in the basal metabolic rate of 2.8; therefore a 10 mgm. dose can be predicted to increase the metabolism from −40 per cent to −12 per cent. The error of prediction is of a slightly less magnitude, if the dosage is based on milligrams for each square meter of body surface. The height

of the metabolic reaction usually occurs on the eighth day. By means of small intravenous doses of thyroxin repeated at short intervals it is known that less than 1 mgm. daily is more than sufficient to replace any loss or destruction of thyroxin in the body, although the exact amount has not yet been definitely determined. However, by extra-

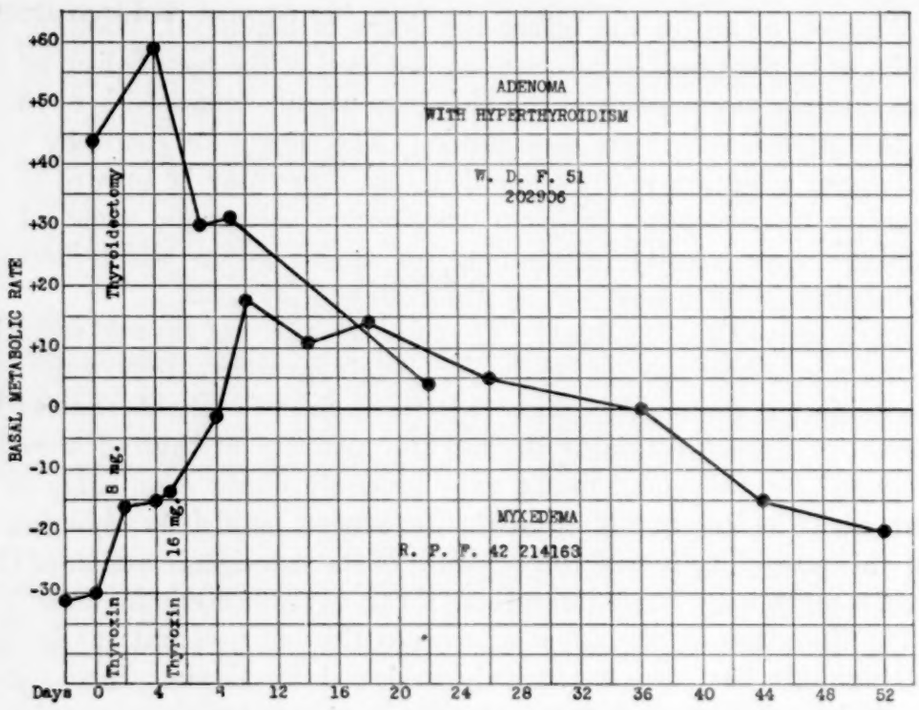

Fig. 1. On the upper curve the basal metabolic rates of a patient having adenoma with hyperthyroidism are plotted showing the original preoperative rate and the immediate postoperative rise in the metabolic rate, followed by the drop to normal after partial thyroidectomy. The lower curve illustrates the effect on the metabolic rate of a myxedematous patient of two intravenous injections of 8 and 16 mgm. of thyroxin, from which can be calculated the amount of thyroxin needed to bring such a patient to a normal metabolic rate, and, therefore, the presumable amount of thyroxin in the body, and also the rate at which the thyroxin is destroyed or eliminated. The daily dose was not started until after the fifty-second day as it was desired to see the duration of the effect of the intravenous medication.

polation of the descending part of the calorigenic curve of thyroxin it can be estimated that the rate of loss or destruction of the thyroxin usually ranges between 0.2 and 0.4 mgm. daily in a myxedematous subject brought up to a normal basal metabolic rate; total metabolism rather than basal metabolism, however, probably governs the rate of

thyroxin loss. From the thyroxin curves it can further be calculated that 12 to 14 mgm. of active thyroxin must be present in the body in order to maintain the basal metabolic rate at the normal level. Pathologic variations in the basal metabolic rate are due, at least in most instances, either to an increase or decrease in this quantity, thus explaining the characteristic and fundamental alterations in the metabolic rate found in hyperthyroidism and in hypothyroidism.

A recent calculation by Boothby and Sandiford of the total excess heat production resulting from the intravenous administration of a known amount of thyroxin shows that for each milligram of thyroxin

TABLE 6

Increase in the basal metabolic rate from intravenous injection of thyroxin in 69 cases of myxedema

	Increase per mgm. 2.8 Average variation 0.7 = 25% Largest plus variation 2.8 = 100% Largest minus variation 1.8 = 64%				Increase per mgm. per sq.m. 4.7 Average variation, 1.1 = 23% Largest plus variation, 2.9 = 60% Largest minus variation, 2.9 = 60%			
AVERAGE BASAL METABOLIC RATE	VARIATION IN CALORIGENIC ACTION DEPENDING ON THE ORIGINAL LEVEL OF THE BASAL METABOLIC RATE				VARIATION IN CALORIGENIC ACTION DEPENDING ON SIZE OF DOSE			
	Average dose	Increase per milligram	Average dose per square meter	Increase per milligram per square meter	Average dose	Increase per milligram	Average dose per square meter	Increase per milligram per square meter
Above to −14	8.7	3.3	5.7	5.0	4.8	3.5	3.6	5.0
−15 to −19	9.1	2.3	5.1	3.9	6.9	2.8	5.2	5.0
−20 to −24	7.3	2.9	4.6	4.6	8.8	2.9	7.0	5.2
−25 to −29	10.9	2.8	6.2	5.0	11.2	3.3	9.3	4.8
−30 to −34	14.8	2.9	9.2	4.9	16.0	2.6	11.5	2.7
−35 and below	15.5	3.0	8.6	5.1	24.0	1.6	13.2	3.0

injected approximately 1008 calories are produced or expressed on the basis of a milligram mole 589,680 calories which would not have been produced had not the thyroxin been given. So far as we know this is the first time that the power of any catalytic agent has been measured in terms of energy; a similar calculation of the catalytic power of adrenalin shows that it is of an entirely different order of magnitude as 1 mgm. of adrenalin produces only approximately 50 calories of extra heat or 9,150 calories on the basis of a milligram mole. This shows that the power or durability of a thyroxin molecule is approximately 64 times that of an adrenalin molecule. The various points of the descending limbs of the calorigenic curves of both adrenalin and thyroxin when plotted on semi-logarithmic paper show a remarkable tendency to lie

upon a straight line; in a large proportion of the experiments the points are within ±5 per cent of such a line and in a few are directly upon it. This is consistent with the idea that the system suffers an essential change of concentration of only one molecular species and that therefore the rate of excess heat production is dependent directly upon the concentration of thyroxin and adrenalin in the tissues.

If thyroxin is given by mouth the height of the metabolic reaction is usually not as great nor as constant as when given intravenously, probably owing to its incomplete absorption from the gastro-intestinal tract. Therefore, the daily oral dosage necessary to maintain the metabolism at a normal level in a myxedematous person is distinctly larger than the calculated rate of elimination given above; the oral dosage is not far from 1.6 mgm. daily.

Aub, Bright and Uridil have shown that the increased basal metabolism stimulated by thyroxin cannot be explained by muscular activity, muscular fibrillation, or increased muscle tonus; and also that the adrenal glands are not essential to the maintenance of the high metabolic rate induced by thyroxin.

Boothby and Sandiford have found in a series of experiments (unpublished) that thyroxin injected intravenously into normal dogs causes a definite and unquestionable rise in the heat production. The reaction, however, is neither as great nor as prolonged in the dog as in the thyroidless human subject. Plummer has shown that a definite reaction is sometimes obtained in patients with exophthalmic goiter following the intravenous injection of thyroxin, at other times little or no effect is produced. The exact explanation of these variations is not known, but may be due as we have suggested to an inverse more or less compensatory decrease in secretion from the thyroid gland.

Kendall, in his fundamental investigations has isolated thyroxin and has synthesized a large series of compounds closely related to thyroxin in chemical configuration. One of these compounds differs from thyroxin in having two less hydrogens and possessing in their place a bond. This bond can be easily replaced by two hydrogens by reduction. This compound therefore is an oxidized form of thyroxin. Kendall believes that both the oxidized and reduced forms of thyroxin are present in the thyroid gland, and the functioning of thyroxin in the tissue is brought about by the molecule of thyroxin oscillating between these two forms and thereby catalyzing oxidation. Expressed in chemical terms, the function of thyroxin is to regulate the capacity of a cell to use oxygen. However, the rate of functioning of thyroxin in the tissues

is dependent on the physical state of the cell. The alternate oxidation and reduction of the molecule is regulated by the opening and closing of the pyrrol ring in thyroxin, and this in turn is dependent on the concentration of positive and negative charges present. It therefore appears probable that thyroxin makes any cell more sensitive to physical forces external to that cell. Kendall therefore has confirmed by a study of the chemical reactions involved the theory of Plummer that "the active agent of the thyroid gland is a catalyst that accelerates the rate of formation of a quantum of potential energy in the cells of the organism." In reference limited to basal metabolism Boothby has stated that thyroxin can be considered as a calorigenic agent which increases the metabolic rate in proportion to the quantity actively present (the concentration) in the cells of the body.

ADENOMATOUS GOITER WITH HYPERTHYROIDISM AND EXOPHTHALMIC GOITER. This is not the place to enter on a discussion of the clinical differentiation of adenomatous goiter with hyperthyroidism and exophthalmic goiter. Suffice to say that the weight of evidence substantiates the clinical differentiation. In both diseases there is an increase in metabolism as shown in table 5. As pointed out by Du Bois, the intensity of the hyperthyroidism is in general proportional to the elevation of the metabolism. Boothby and Sandiford have shown that contrary to the general belief the endogenous nitrogenous metabolism per se is not increased in these diseases and that, therefore, it cannot be the cause of the increased basal metabolism.

In adenomatous goiter with hyperthyroidism the increase in basal metabolism is slow and insidious but usually definitely progressive. The clinical symptoms are those due at least in great part to the maintenance, for months and years, of an increased basal metabolism on which in the later stages is superimposed the evidence of organic overwork, the most characteristic of which is cardiac failure. We have reproduced the symptoms (except those of degeneration) in their entirety by the administration of excess of thyroxin over several months. The conclusion seems warranted that the symptoms of adenomatous goiter with hyperthyroidism are due to the presence in the cells of the body of an increased quantity (concentration) of thyroxin, and that the evidence substantiates the conception that not only is the thyroxin a normal product but that the reactions through which it passes are proceeding in a normal manner without a relative increase in the quantity of any intermediate product.

Plummer has shown that the symptoms of exophthalmic goiter cannot be fully explained on the foregoing simple conception, largely because there are two recognizably distinct syndromes. For many years Plummer has believed that in exophthalmic goiter the secretion of the thyroid is not only increased but is also somewhat imperfect, or that the series of reversible reactions into which it entered are not proceeding normally. The recent investigations of Kendall support this view strongly, as he has been able to synthesize many members of the same

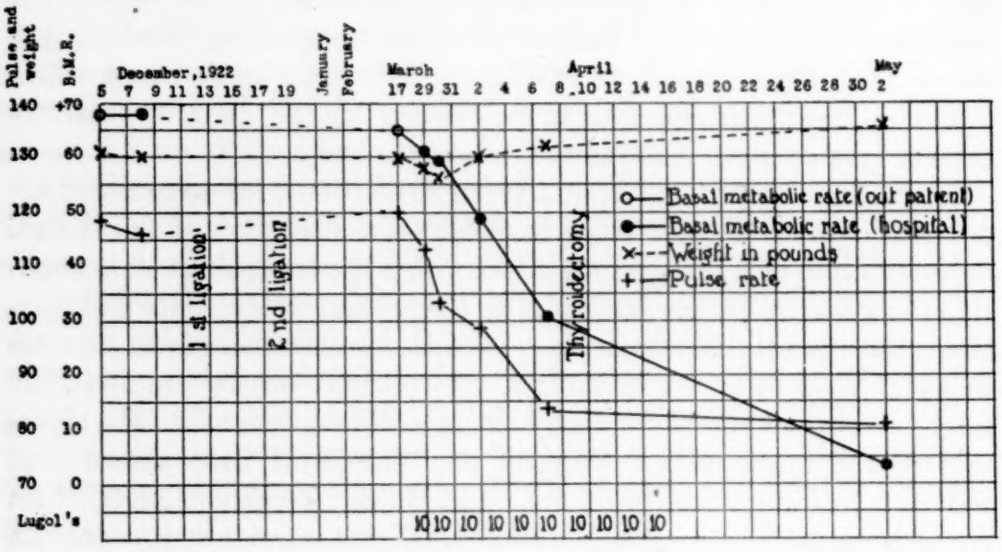

Fig. 2. Case A411837 F. Aged 30. Thyroid: both lobes 2.5 by 2.5 inches. Thrills 2+; bruit 2+; exophthalmos 2; heart 1 by 3.5 cm.; no murmur, heaving; slight edema of the legs; loss of strength; normal weight 138 pounds. Duration of goiter 2.5 years; symptoms about one year. No crisis. This figure illustrates the constancy of the basal metabolic rate over a period of three months. It was essentially unaffected by ligations and rest. Following the beginning of the treatment with Lugol's solution, 10 drops daily, there was an immediate and marked decrease in the basal metabolic rate from +65 to +31 before thyroidectomy followed by a further drop to normal after operation.

chemical family. Therefore a year ago last March, Plummer attempted on a priori grounds to modify the symptoms of exophthalmic goiter by giving iodin in large doses. *Liquor iodii compositus* (U. S. P.), known as Lugol's solution, was selected because it contains, a large quantity of iodin in aqueous solution

During the past year Plummer has reported at several meetings the marked effect in exophthalmic goiter of large doses of iodin on lowering the metabolism, decreasing the severity of the clinical symptoms and

preventing the post-operative thyroid crisis which so often resulted in death. The clinical phase of the subject need not be entered into here. The point of interest to the physiologic chemist is the fact that by the administration of iodin a striking, unquestionable and profound alteration is produced in the metabolic processes dependent upon the thyroid secretion in exophthalmic goiter. This is evidenced in part by a marked

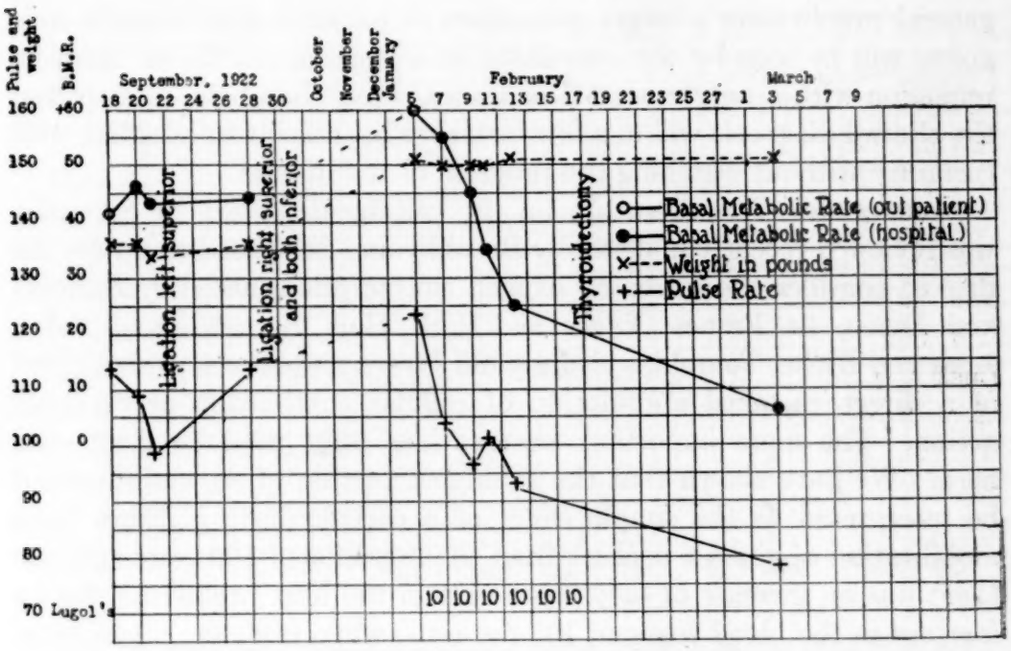

Fig. 3. Case A404976, F., aged 33 years. Right lobe to thyroid 1.75 by 2.5 inches, left lobe 1.25 by 1.75 inches. Bruit 2; thrill 2; exophthalmos 1+. Heart 2.5 by 11 cm. Systolic murmur at apex. No edema. Marked dyspnea; loss of strength 2+. Normal weight 155 pounds. Duration of goiter five months; symptoms eight months. This figure is another typical illustration of the effect of Lugol's solution on the level of the basal metabolic rate in exophthalmic goiter. The metabolism was higher instead of lower subsequent to ligations and rest for five months. The promptness of the drop from +60 to +25 after Lugol's solution (10 drops daily) was started seems unquestionably due to iodin.

decrease in the basal metabolic rate. The accompanying figures (figs. 2 and 3) illustrate the typical influence of iodin on the basal metabolic rate. In a few cases only does clinical improvement occur with only a slight lowering of the basal metabolism. Not one of our cases of exophthalmic goiter has been rendered worse or the metabolism increased as a result of iodin. The conception that it might do so originated probably from the well-known fact that adenomatous goiter which

is not hyperfunctionating, without constitutional symptoms and with a normal metabolism, can often be transformed into adenomatous goiter with hyperthyroidism with the typical symptoms of that disease including an increased basal metabolism, by the administration of iodin; this condition has been referred to as Jod-Basedow by Kocher and others and erroneously interpreted as exophthalmic goiter initiated by iodin. On the other hand in the future as a result of the use of iodine by the general practitioner a larger proportion of patients with exophthalmic goiter will be seen by the consultant in a stage of partial or complete remission with a nearly normal or normal basal metabolic rate so that the clinical diagnosis will in a few instances be difficult to establish with certainty without watching the progress of the disease.

ADRENALIN. The literature on the calorigenic action of adrenalin was reviewed recently in detail by Boothby and Sandiford who were the first to consider the increased oxygen consumption, noted by Barcroft and Dixon, La Franca, Fuchs and Roth, Hari, Bernstein and Falta, Lusk and Riche, Tompkins, Sturgis and Wearn and others, as significant of a direct chemical stimulation of cellular combustion or catalytic action. The more important observations only need be mentioned here. We have shown that the calorigenic action of adrenalin cannot be interpreted as the simple effect of a carbohydrate plethora from mobilization of glucose because from the ingestion of 100 grams glucose there was an increase of only 9 per cent in the heat production for an increase in the blood sugar of 105 per cent, while from the injection of 0.5 mgm. adrenalin there was an increase of 20 per cent in the heat production for an increase of only 37 per cent in the blood sugar. They also obtained a marked reaction in a severe diabetic. Boothby and Wilder have shown that the calorigenic action of adrenalin is unaltered even in the presence of a rapidly falling blood sugar as the result of the administration of insulin.

Aub and Bright showed that in the absence of the adrenal glands, adrenalin injected intravenously at the rate of 0.004 mgm. each minute in cats caused an increase in heat production.

That the intravenous injection in the intact, healthy dog of doses of adrenalin as small as 0.0006 mgm. for each kilogram each minute, will produce a definite and positive calorigenic action, has been proved by Boothby and Sandiford, and as a result they conclude that one function of the adrenal glands is to furnish a secretion which accelerates the velocity of an as yet unknown chemical reaction, or reactions, in the direction accompanied by the liberation of heat. As will be referred to

in the discussion of insulin, this calorigenic action of adrenalin is so definite as to be useful in directing attention to a possible mechanism, hitherto unsuspected, associated with a probable spontaneous discharge of epinephrin which may be concerned in the prevention of hypoglycemia.

From the calorigenic curves of adrenalin, a typical curve being shown in figure 4, we have calculated that from the subcutaneous injection of

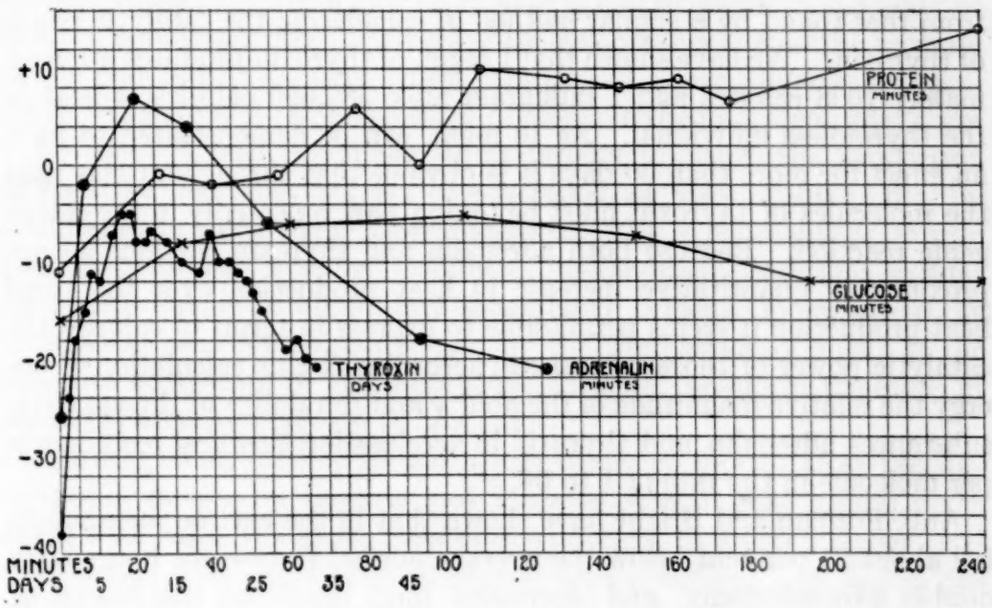

Fig. 4. The time elapsing after the administration of the drug is plotted in minutes for protein, glucose and adrenalin, and in days for thyroxin. The amounts administered were as follows: protein and glucose 100 gm. each in a normal male subject, aged thirty-four years, weight 70 kg. and height 168 cm.; 0.5 mgm. adrenalin subcutaneously in a normal male subject, aged thirty years, weight 61 kgm. and height 173 cm.; 10 mgm. thyroxin intravenously in a case of typical myxedema in a man aged thirty-six years, weight 76 kgm. and height 171 cm.

1.0 mgm. adrenalin approximately 50 calories and from one milligram mole 9150 calories of heat are produced in excess of what would have been produced had not the adrenalin been given. The reaction ceased presumably because the adrenalin was gradually destroyed; although each molecule of adrenalin may have entered the reaction only once it seems probable that in order to produce 50 calories of energy it must have taken part repeatedly in accelerating a reaction accompanied by the liberation of so much heat. Boothby and Vidlicka have

shown that the quantity of heat (50 calories) so produced by each milligram of adrenalin with the subject at rest in bed remained practically unchanged when the subject carried out various measured degrees of light and moderate work by walking on a treadmill. In one experiment with severe work approximately twice as great a reaction was obtained; deductions, however, based on this single experiment are not justified until it is shown whether or not this increase was accidental. The catalytic power of adrenalin is of an entirely different order of magnitude from that found by a similar method of calculating the catalytic power of thyroxin. We have shown that 1 mgm. of thyroxin produces approximately 1008 calories and 1 milligram mole 589,680 calories of heat in the thyroidless individual. As 10 mgm. can be demonstrated to have an effect for more than 30 days it is obvious that throughout this time the molecules of thyroxin must be taking part repeatedly in the calorigenic reaction. Because both adrenalin and thyroxin produce an approximately quantitative increase in heat production over the basal heat production it seems permissible to express in terms of calories the catalytic power of those substances; according to this calculation of energy the relative magnitude of the ability and durability of the catalytic substances adrenalin and thyroxin in accelerating chemical reactions is per molecule in the rate of 1 to 64.

Aub, Forman and Bright have shown that in the cat the metabolism fell about 25 per cent below the average normal forty-eight hours after double adrenalectomy, and decreased until death on the fourth or fifth days. Aub, Bright and Forman also found evidence tending to indicate that the removal of the adrenals caused a prompt and progressive fall of the metabolism with the cat under urethane anesthesia. Marine and his associates have presented a series of papers which appear to indicate that there is a primary increase in heat production after removal or crippling the adrenal glands by freezing in rabbits with intact thyroids. The inconstant results which they obtain from freezing they explain on the ground that if a decrease in metabolism is obtained the injury is too severe and if no change is obtained the injury is too slight. They also find thyroidectomy prevents the increased heat production and therefore draw very far-reaching conclusions about thyroid-adrenal interrelationships in the causation of Graves' disease.

Insulin. Between 1913–1916 Murlin, Kramer and Sweet in Lusk's laboratory carried out a series of experiments on dogs made totally diabetic by removal of the pancreas which showed evidence of utilization of sugar by a rise in the respiratory quotient following the administra-

tion of pancreatic extract. Some of these experiments were reported in a series of papers published at the time while others were not published until 1922 following the reports of Banting and Best on the discovery of insulin. Recent papers by Murlin and his associates from the University of Rochester showed that in the depancreatized diabetic dog, insulin, or rather various types of pancreatic extracts and perfusates raises the low respiratory quotient characteristic of this condition by several points provided a glucose reserve is still available in the body or is given simultaneously. Several of their experiments show no increase in the respiratory quotient when sugar is not administered simultaneously. A failure of the respiratory quotient to rise may mean that there is not enough sugar available to materially influence the proportion of sugar burned; this fact can be controlled, as Murlin usually did, by simultaneous blood sugar determinations. The experiments of Murlin and his associates were not conducted under basal conditions so that they were unable to obtain any satisfactory evidence in regard to the increase in heat production caused by the combustion of sugar or the presence or absence of any calorigenic action of insulin in the sense of that exhibited by thyroxin or adrenalin.

Banting, Best, Collip, Hepburn and Macleod shortly after the discovery of insulin by Banting and Macleod showed that insulin given along with sugar raised the respiratory quotient to a much higher level than occurs with sugar alone in several depancreatized dogs and in one patient. The heat production was not noted. Two similar experiments were also reported by Banting, Best, Collip, Campbell, Fletcher, Macleod and Noble before the Association of American Physicians. Confirmatory experiments on the respiratory quotient are also mentioned incidentally in several other publications by the Toronto group.

Boothby and Wilder have reported four experiments on the effect of insulin on the heat production in normal and diabetic individuals under standard post-absorptive conditions. In one diabetic they found as a result of administering twenty units of insulin a fall in the blood sugar during three hours and twenty minutes from 286 to 100 mgm.; during this time repeated determinations of the basal metabolic rate remained essentially constant, the several determinations being −17, −18, −20, −17 and −22 per cent. When the blood sugar reached 100 mgm. there was a sharp rise in the metabolism to −10 per cent. At first the respiratory quotient rose slightly from 0.75 to 0.82, 0.82, 0.85 and 0.81 indicating that the excess sugar present was converted into a form which would burn; this rise was later followed by a decrease to 0.79 as the blood sugar

dropped to 100 mgm. and the metabolism rose. The other three experiments likewise showed that insulin per se does not directly increase the rate of heat production. Besides these acute experiments they reported that the daily administration of insulin in many patients over long periods did not produce any demonstrable effect on the level of the basal metabolic rate, provided the diet remained essentially constant and there was no change in the nutritional status of the subject. They concluded therefore that insulin does not itself possess an immediate calorigenic action like adrenalin nor a delayed calorigenic action like thyroxin.

There was, however, an increase in the metabolism as the blood sugar fell to what for that individual was the critical level for the symptoms of hypoglycemia. Boothby and Wilder pointed out that the increase in metabolism at this point seemed to be secondary to the low level of the blood sugar, or rather to the mechanism that altered the direction of the drop in the blood sugar curve when the latter reached its critical level. At this point the shape of the metabolism curve and other phenomena observed were so similar to that after the administration of adrenalin that the possibility of a spontaneous discharge of epinephrin was considered. In a footnote to their paper on the calorigenic action of adrenalin, Boothby and Sandiford refer to this reaction and to the fact that Cannon, McIvor and Bliss have presented additional evidence supporting the view that the above reaction may be indicative of an automatic discharge of epinephrin as a protective measure against hypoglycemia.

The function then of insulin appears to be limited to converting glucose into a form of carbohydrate that can be directly utilized by the cells according to their needs and not to be concerned with increasing or decreasing the rate or proportion of carbohydrate burned except in the sense suggested by Krogh and Lindhard for the normal individual "that both during rest and during work the proportion of fat to carbohydrate catabolized is a function of the available supplies of these substances." In the diabetic organism the proportion of available carbohydrate must necessarily refer to sugar in an active form available for transformation into energy without further action of insulin. If both glucose and insulin are in excess of the needs there will be an ample supply of the combustible carbohydrate while if either one is lacking there will be a dearth of sugar in an active form. Under these circumstances, according to the theory of Krogh and Lindhard, the process of fat changing into this active carbohydrate will be stimulated.

Results on dogs and rabbits quite similar to those described above for man were reported at the December (1923) meeting of the Federation of American Societies for Experimental Biology by Dickson, Eadie, Macleod and Pember as follows: Respiratory experiments on dogs and rabbits following the injection of insulin show: *a*, in dogs an early rise of the respiratory quotient during the time when the blood sugar is rapidly falling, succeeded by a marked fall coincident with an enormous increase in ventilation, oxygen consumption, and carbon-dioxide excretion, preceding and culminating in convulsions. At the same time the respiratory and pulse rates increase and the temperature falls. If by giving glucose the blood sugar is prevented from falling below 80 mgm. per 100 cc. we find the early rise in respiratory quotient noted before with a secondary rise some time later, but the increases in ventilation, oxygen consumed and carbon-dioxide excreted, etc., do not occur. *b*, In rabbits there is the early rise in respiratory quotient and the subsequent fall, but the other changes which precede convulsions in dogs do not occur.

The available evidence is against the rise of respiratory quotient's being due to a "blowing-off" of carbon dioxide either from hyperpnea (since it occurs distinctly before the hyperpnea which is accompanied by low quotients) or increased acid production (studies on blood and urine published elsewhere). There is no direct evidence of the conversion of glucose into a fat-like body.

The later rises in ventilation, etc., are thought to be due to hypoglycemia, probably acting through the central nervous system, exciting increased respiration and muscular tone.

DIABETES. There has been an interesting discussion between Lusk, Du Bois and their associates and Benedict and Joslin whether or not the metabolism was increased in diabetes. In 1910 and 1912 Benedict and Joslin maintained that in severe diabetes the basal metabolic rate is increased. Allen and Du Bois, however, were able to show that this conception arose in the main from an improper selection of standards and that they were only 2 per cent above the Du Bois standards. There was, on the other hand, some truth in the contention of Benedict and Joslin, which has since been brought out, because the metabolism in the patients observed by them was higher than in undernourished normal individuals of similar size and age and also higher than in Joslin's patients later treated in accordance with Allen's method of under nutrition.

Wilder, Boothby and Beeler in a study of thirty-one patients with diabetes threw further light upon the subject by showing that the

method of feeding and degree of under-nutrition of the patients materially influenced the level of the metabolism. They found the basal metabolic rates to be within 10 per cent of the Du Bois normal standards in ten out of twelve uncomplicated cases without emaciation; the other two had increased rates. The patients with body weights 20 per cent below standard with uncomplicated diabetes had with only one exception a depression of their basal metabolic rates from −11 to −32 per cent, the degree of depression being possibly roughly proportional to the degree of loss of weight below the standard. Two emaciated patients had rates of +3 and +15 per cent respectively on the two or three days following their admission to the hospital. Both of them had been eating a large amount of food including liberal quantities of protein; in each case the rate promptly decreased on the institution of low diets. The well nourished and the obese patients, although many of them had fallen in weight below their previous maximal weights, did not show any depression of their basal metabolic rates despite dietary deficiencies. Presumably obese persons maintain their metabolism readily at the normal level on low diets at the expense of calories derived from endogenous sources. They pointed out that in diabetes several factors influenced the level of the basal metabolic rate: *1.* In the undernourished patient the basal metabolic rate was below normal if he was on a maintenance diet equal to the basal requirement, plus a small allowance for work, with the protein quota not exceeding 1 gram per kilo. *2.* In these undernourished individuals the ingestion of food containing a small amount of protein but with an excess of fat and carbohydrate considerably above the maintenance requirement caused an elevation of the basal metabolism. *3.* The ingestion of an excess of protein (3 grams or over) caused in some patients a greater rise than occurred with isocaloric amounts of other foods. *4.* In well-nourished patients the basal metabolic level does not exceed the normal limits of Du Bois' standards unless the patient is on a high protein or high caloric diet or has a complicating condition such as an infection or hyperthyroidism. *5.* Acidosis itself did not appear to elevate the basal metabolic rate because, in the case carefully studied by them and in which the time relationship of the various facts was accurately known, the basal metabolism and D to N ratio increased twelve to twenty-four hours in advance of the acidosis. Therefore, they considered the increase in rate accompanied by an acidosis as probably due to the factor that caused the acidosis.

One other factor which may and probably does affect the level of the basal metabolic rate hitherto unconsidered in diabetes is the loss of

energy which Krogh and Lindhard have shown to occur when fat is converted into a carbohydrate-like substance. This substance is apparently combustible in the diabetic organism because no evidence of its formation is obtained in the urine: therefore it enters the series of intermediary reactions below that upon which insulin acts. Acidosis has long been considered as evidence that the body is not burning carbohydrate in normal quantities and in diabetes is primarily due to a lack of insulin (natural or artificial). Acidosis, however, develops in normal individuals in whom there is no lack of insulin but in whom the glycogen reserves have been consumed and all energy must come from fat or protein. It is possible, therefore, that acidosis, accompanied by a slight elevation in the metabolism is an indication that fat is being transformed into this combustible carbohydrate substance at a rate greater than normally occurs when carbohydrate is ingested in normal amounts in the presence of a normal concentration of insulin. Conversely, the reaction in which acetone bodies are formed may be responsible, at least in part, for an indirect as well as direct excess or waste heat production.

Joslin, in his recent monograph on metabolism deals at length with the level of the basal metabolism in diabetes. Some of the particularly high values which he reports are due to the fact that he uses the Harris and Benedict standards, fully discussed in this paper under the section devoted to standards. Joslin's main point is that in the patients studied before June, 1914, the average variation from the Harris and Benedict standard was +12 per cent and after this date was −11 per cent. In his book on treatment he points out that prior to June, 1914 his diabetic patients were overfed with a low carbohydrate and high protein-fat diet and an excess of calories was encouraged. After that date the Allen system of treatment based upon a reduced diet was adopted. His observations, therefore, are analogous to those of Wilder, Boothby and Beeler as to the practical advantages of a low protein diet with the calories limited to the maintenance requirement. As to the theoretic cause of the increased metabolism Joslin argues that it is essentially caused by acidosis and asks if it is justifiable for Wilder, Boothby and Beeler to ascribe the entire rise to protein and disregard the fat with its capacity for forming acid bodies? As pointed out on the preceding page this is exactly what Wilder, Boothby and Beeler did not do; they state that in their opinion it was not the acidosis itself which caused the rise in metabolism but the factors which produced the acidosis. They were careful to emphasize that not only excessive protein but also

excessive fat or excessive calories were among such factors. They did state, however, and Joslin has offered no evidence to contradict it, that the sugar tolerance of a patient is much more markedly depressed by an excess of protein than by an isocaloric amount of fat.

Joslin has collected the recent reports of low respiratory quotients in diabetes which appear to be trustworthy, and points out that they are, at least to a certain extent, like the dextrose-nitrogen ratios of 3.65 to 1, forced quotients and forced ratios and that they do not occur in the undisturbed diabetic patient. He does not consider them inherent in the ordinary course of diabetes but that they occur only as the result of accident or design. The average respiratory quotient in his series before June, 1914 (on low carbohydrate and high protein-fat diet with over-feeding) was 0.73 and after June, 1914 (on Allen's fasting and under nutrition method) was 0.78. Joslin points out that the respiratory quotient in diabetes appears to have a definite relation to the metabolism in that it is low when the metabolism is high and higher when the metabolism is low. Although he considers a high respiratory quotient welcome in diabetes, because it suggests the ability of the patient to burn carbohydrate, he emphasizes that it is obtained only through a metabolism of threateningly low degree. We must point out, however, that if the same quantity of carbohydrate is being burned in either case the lowering of the total metabolism will give a rise in respiratory quotient simply as a mathematical result, and does not indicate therefore any improvement in the ability of the patient to burn carbohydrate; this explains Joslin's opinion that a high quotient is not necessarily a good clinical sign. Joslin considers that the low respiratory quotients in diabetes which have been obtained under carefully controlled conditions in the special metabolism laboratories and have been regarded with suspicion by them as to their technical accuracy must not be dismissed from further consideration; he concludes that they are forced quotients due to ingestion of much protein and fat with little carbohydrate, but offers no concrete suggestion as to what they may signify, although he does refer indefinitely to the possible application of the hypothesis of Krogh and Lindhard. As these low quotients will be discussed by us in another section we will not stress their significance here. On the other hand Joslin believes that there is some evidence in the extremely high quotients which he found to indicate that these occurred when lack of available fat necessitated unusual catabolism of body protein without simultaneous oxidation of the protein residue, or even transformation of carbohydrate into fat. However he presents no evidence to substan-

tiate the assumption that the protein residue was not oxidized in the usual manner. His suggestion that carbohydrate can be converted into fat when it cannot be burned is interesting, although it appears to be contrary to any quantitative studies we have at present in regard to the carbohydrate balance in diabetes.

THE BASAL METABOLISM AND RESPIRATORY QUOTIENT IN RELATION TO A POSSIBLE CONVERSION OF FAT INTO A CABOHYDRATE-LIKE SUBSTANCE. The method of calculating the respiratory quotients of the various foodstuffs is admirably presented by Lusk, both for the normal combustion, and for diabetes, and need not be repeated here. For normal combustion the accepted values of the respiratory quotient can safely be taken for fat as 0.710 ± 0.003; for protein as 0.80 and for carbohydrate as 1.000. The quotient for protein is probably the least accurate, as the figure represents the resultant of the oxidation of many different proteins, the composition of which is not known although they are composed of varying proportions of amino-acids, the quotients of which do not vary greatly from 0.80. In the diabetic individual with a D to N ratio of 3.65 to 1 the quotient from protein is 0.632 because 58 per cent of the protein molecule is eliminated in the urine as glucose.

Lusk points out very emphatically the danger of attaching too much significance especially in diabetes to quotients that are apparently slightly below 0.71, and of attempting to utilize them as evidence for the conversion of fat into carbohydrate. He shows that the respiratory quotient for fat might be apparently reduced from 0.707 to 0.669, if the maximal quantity (36 grams) of oxybutyric acid is formed from the combustion of 100 grams fat; on the other hand he also shows that the matter is not so simple, because if sodium bicarbonate is utilized to neutralize the oxybutric acid, the apparent quotient is thereby again raised to 0.715, and that if other substances are utilized for neutralization, a different result might be obtained.

Lusk's statement, that the theory of the conversion of fat into glucose has been shattered, and that it may be relegated to the realm of scientific superstition, has served a very useful purpose in limiting speculation as to the significance of very low quotients, many of which were without question due to improper technic.

Since the foregoing statement was made the whole problem has passed into a very different phase. Meyerhof and A. V. Hill with Fletcher and Hopkins have shown that probably muscular contraction itself is not an oxidative reaction, but is due to the splitting of a definite unknown molecule apparently closely related to the carbohydrates with the forma-

tion of lactic acid; the reconversion of lactic acid back into this unknown substance is, however, an oxidative reaction. By very exact methods these investigators have succeeded in obtaining a balance sheet of the substances involved and the amount of heat produced which most strongly support their deductions. If, as they maintain, muscular contraction is due to the decomposition of a molecule of a carbohydrate character it is improbable, in conditions of prolonged fasting or exclusive fat diet, that the quantity of heat evolved in muscular work can be accounted for on the basis of the carbohydrate derivable from protein, or the storage capacity of the body for glycogen. It seems more probable that by some series of reactions there is a conversion of fat into this carbohydrate-like substance. The chief argument against the probability of such a reaction has lain in the evidence in diabetes that no sugar is eliminated in the urine the origin of which can be traced to fat, certainly none aside from the glycerole fraction. Our recent advance in knowledge of diabetes and the probable rôle of insulin, diminishes the value of such evidence as it can be readily assumed that the carbohydrate-like substance formed is beyond the step in the series of intermediate reactions which require insulin for their proper progression and that the substance thus formed can be utilized by the diabetic organism and, therefore, is not excreted. This conception is fundamentally different from that advanced by Geelmuyden in his recent article in Ergebnisse der Physiologie (1923).

By a very brilliant series of experiments Krogh and Lindhard have shown that the loss of energy in work on a fat diet is of a definite magnitude. In their three best experiments they found the waste of energy from fat to be 11 per cent of the heat of combustion of fat. They agree, however, with Zuntz in denying a loss of 30 per cent, as suggested by Cheveau, or of 24.4 per cent, according to a calculation by Zuntz. Krogh and Lindhard showed also that on the transition from rest to moderate work the respiratory quotient is generally altered. On the average it was increased when the preliminary rest quotient was low, and diminished when it was high before the work. The fall at high quotients is greater (0.05) than the increase at low (0.03). At quotients between 0.8 and 0.9 the average change in transition to work is very slight. They suggest as a working hypothesis that:

1. Both during rest and during work the proportion of fat to carbohydrate catabolized is a function of the available supplies of these substances;

2. Carbohydrate is formed from fat and provisionally stored when the quotient is below 0.8, while a corresponding transformation of carbohydrate to fat takes place when the quotient is above 0.9;

3. These anabolic processes make the total respiratory quotient lower than the catabolic when this is low, and higher when it is high, and they give rise to an extra expenditure of energy during rest;

4. During work the anabolic processes (combined with storage) are not increased in proportion to the catabolic, whereby the total quotient was lowered when it was high and raised when it was low beforehand.

In the publication of Wilder, Boothby and Beeler previously referred to the average respiratory quotient, based on forty-four tests, was found to be 0.693, and the non-protein respiratory quotient, 0.679 in a case of severe diabetes. They were very conservative in their consideration of the significance of these quotients and stated that the variation from 0.71 was probably due to the following factors: *1*, temporary alteration in the character of the respirations so common in all short period experiments; *2*, variation in carbon dioxid carrying power of the blood, depending on whether acidosis is increasing or diminishing at the time of the test; and *3*, utilization of oxygen and carbon in the formation of acetone bodies. They were not, therefore, inclined to read into their data evidence of the formation of sugar from fat. A review of the data, however, reveals interesting facts not brought out at that time. The experiment lasted seventy-four days; during that time the patient ate carbohydrate 1147 grams, protein 3170 grams, and fat 7106 grams, with an elimination of 2206 grams of sugar in the urine. The total glucose value of the foregoing diet is 1491 grams, adding 58 per cent of the protein, 10 per cent of the fat and the ingested carbohydrate minus the amount eliminated in the urine. This 1491 grams of glucose could furnish 6113 calories of carbohydrate for muscular work. The patient's basal calories, however, for this period totalled 68,746. The total heat value of the food, less that eliminated in the urine, was 74,744 calories, which is approximately 9 per cent greater than the calculated basal heat production and, as the patient's weight remained constant, this must very closely approximate the total metabolism. The difference between 74,744 and 68,746 calories which equals 6000 calories is, then, the effect of the specific dynamic action of the food and the extraneous muscular work. As part of the specific dynamic action can be offset by the decrease in metabolism during sleep, we may assume that at least two-thirds of this or 4000 calories is due to extraneous muscular movement in bed and around

the room; at least an equal amount or approximately 5 per cent of the total metabolism must be assumed to be due to muscular work of respiration, heart and smooth muscle, so that a total of 8000 calories can be considered as being produced directly by muscular contraction; as found above there were only 6113 calories for this purpose, assuming that none of it was used otherwise. Can the balance of 1887 calories or 460 grams come from stored carbohydrate and if so how long could such conditions be maintained or is it without significance on account of the small deficiency found?

Benedict, using his respiration apparatus, calculates the basal requirement of his fasting man, when lying awake in the morning, as 38,845 calories for the thirty-one days, which is considerably more than was obtained in the calorimeter when the subject was asleep. Therefore, the difference between the metabolism with the subject awake in the morning, and the calculated total for the day, must represent almost entirely muscular movement. He estimates the total metabolism as 46,166 calories. The balance 7321 calories is then due to muscular work without any consideration of the muscular work of the heart and respiratory muscles, which if assumed as 5 per cent of the total (46,166 $\times$ 0.05) would make 2308 + 7321 = 9629 calories that were used in muscular contraction, or an equivalent of 2349 grams of carbohydrate. It seems unlikely that this amount could have been stored in the body or obtained from protein and glycerol of fat even if all of it be reserved exclusively for muscular work as opposed to the basal requirements. During this period there were 277 grams nitrogen in the urine or 1731 grams protein which would yield 1004 grams carbohydrate (1731 $\times$ 0.58) and an additional 365 grams could be assumed to come from the 3650 grams fat or a total of 1369 grams carbohydrate, computed by the ordinary methods of calculation. As the amount of carbohydrate estimated as necessary is 2349 grams there remains practically 1 kgm. (2349 $-$1369 = 980 grams) of the carbohydrate substance used in muscular work to be obtained from some source which our usual assumptions do not include. Are we not forced to at least take into consideration the possibility of an unknown transformation of fat into carbohydrate in the sense suggested by the experiments of Krogh and Lindhard? In this connection it is interesting to note, furthermore, that the respiratory quotient throughout the fast on the respiration apparatus was invariably from 0.02 to 0.05 point higher in the morning (average of 0.732) after a possible conversion and storage of carbohydrate during the night, than in the evening(0.702), and that the respiratory quotient obtained in the calorimeter

during the night (0.717) was nearly always between the extremes found by the respiration apparatus in the morning and in the evening.

Richardson and Mason find, by calculation, a negative carbohydrate balance, using the respiration calorimeter in a very careful and detailed study of diabetes. "It means that to the diet as given the tissues may contribute a quantity of glucose which is, in the absence of respiratory data, unknown.—Another interesting question arises as to its source. Certainly it cannot come from the original store of glycogen present in health, which is estimated by Joslin to average 400 grams and is hardly sufficient to last the month out at the rate at which we have observed it to be withdrawn." In their calculations they have allowed for the carbohydrate formed from protein, hence the large negative carbohydrate balance remains unaccounted for. They are very careful, however, not to suggest the possibility of a formation from fat.

Carpenter has shown that in normal persons there is a drop in the respiratory quotient during sleep. According to the theory of Krogh and Lindhard, this would indicate that while the subject is at rest and asleep there is a building up of a reserve of a carbohydrate substance, and that it makes itself evident mathematically in the quotient by the depression of the total catabolic processes which occur during sleep.

The calculations presented in this section were made by us to see whether or not the evidence at hand did or did not support the idea that fat can be converted into sugar according to the working hypothesis recently advanced by Krogh and Lindhard. We publish them not as proof of this theory but to stimulate others, as it already has us, to plan investigations that will help to solve correctly this important question. Therefore, throughout this paper as occasion presents we have not hesitated to direct attention to the possible applicability of the fat-carbohydrate reaction.

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